A. COMPONENT COVER PAGE

Project Title: Project 1.2 Identification and Development of Anti-Flavivirus Lead Drug Candidates

Component Project Lead Information:

Diamond, Michael S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Project 1 is designed to identify and characterize small molecule inhibitors of flaviviruses, a family of single stranded, positive sense RNA viruses that are associated with significant worldwide morbidity and mortality. This proposal builds on existing expertise in small molecule screening for DENV and is designed to identify small molecule compounds with the potential to be developed as antiviral agents. The initial screen in this proposal will focus on two medically relevant flaviviruses: dengue viruses (DENV) and West Nile virus (WNV). An existing screening platform will be adapted to screen multiple compound libraries, which include a high representation of nucleoside and nucleotide analogs, potentially compounds that have activity against multiple flaviviruses. If broad-spectrum leads with efficacy against multiple viruses can be identified, their further development will be emphasized. In order to enrich for potentially broadly acting compounds, we will focus on compounds that target one of the following important enzymatic activities of the flavivirus NS5 protein: the RNA-dependent RNA polymerase (RdRp), which is essential for replication of the viral RNA genome and the 2'-O-methyltransferase (2'-O-MTase), which is required for the virus to evade the host innate immune response. These activities are conserved among the flaviviruses, and similar activities are found in other virus families as well. The overall CETR proposal contains several projects focused on various virus families that are linked by a central screening facility and compound libraries. Therefore, the parallel screening strategies will maximize the likelihood of identifying broad-spectrum antiviral agents that may function across multiple virus families. The specific aims of Project 1 are:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds identify novel inhibitors of flavivirus replication.
Rationale: The Southern Research Screening Core (SR SC) has developed and validated cell-based, high-throughput assays for inhibitors of DENV and WNV induced cytotoxicity. Initial use of this, or similar, assays has already identified several compounds with antiviral activity. Therefore, this assay will be used to screen novel libraries that have not previously been extensively screened against human pathogens.
Experimental strategy: A CPE based assay will be used as a primary screen for compounds with anti-DENV or anti-WNV activity. Additionally, the WNV screen will be modified in order to allow the detection of compounds that inhibit the viral 2'-O-MTase, thereby sensitizing the virus to the actions of interferon and its effectors. Following the initial screen, "hits" will be evaluated in dose response and cytotoxicity assays in order to determine EC50, CC50, and selective indexes.
Aim 2: Characterize the antiviral activity of hit compounds
Rationale: Hit compounds will be further characterized with regard to efficacy and mechanism of action. The primary screen will potentially identify compounds that inhibit any of the stages of the viral replication cycle, therefore, secondary experiments are designed to elucidate the stage at which individual compounds act. Additionally, we will also characterize the compounds with regard to breadth of activity against other viruses, and examine the potential for evolution of compound-resistant mutants.
Experimental strategy: We will initially test compounds against sub-genomic viral replicons, which will identify compounds that do not function through affecting viral entry or egress, allowing us to focus on inhibitors of translation, protein processing, or RNA replication. We will also identify compounds that function through inhibition of the 2'O MTase, as well as compounds that act non-specifically through induction of interferon or other innate pathways. Compounds will also be evaluated in viral growth assays in order to evaluate the their effect on inhibition of production of infectious progeny virus. Additionally, we will analyze compound effects against multiple viruses and in multiple cell types. Finally, we will test the ability of the virus to develop resistance to individual compounds, as well as characterize any such mutants.
Aim 3: Chemical optimization and in vivo efficacy of lead compounds in animal models of West Nile and Dengue infection.
Rationale: Hit compounds identified and characterized above will be optimized to increase efficacy, selectivity, and bioavailability. These compounds will progress to testing in mouse models of infection.
Experimental strategy: Specific compounds and scaffolds will be triaged by the Medicinal Chemistry and Lead Development Core (MCLDC). Compounds with appropriate pharmacokinetic properties will be tested for prophylactic and therapeutic effects in mouse models of WNV and DENV infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B 2 Wash U.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Wash U.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

With the completion of a HTS by SRI to identify small molecule lead candidate hits that specifically target 2'-O methyltransferase activity, the Diamond laboratory will validate such hits by performing full multi-step growth curve analysis in parallel with cytotoxicity studies. This will allow a selected subset of compounds to be tested against other cell types and flaviviruses using WT viruses in cells lacking or expressing IFIT1.

B.2. Progress on CETR project.

A. Rationale. 2'-O-MTase as a target for small molecule screens. Type I interferon (IFN) cell-intrinsic antiviral defenses protect against many virus infections by signaling host blockade of viral translation, transcription, and replication, thus limiting spread and pathogenesis. Cellular mRNA of higher eukaryotes and many viral RNA are methylated at the N-7 and 2'-O positions of the 5' guanosine cap by specific nuclear and cytoplasmic MTases, respectively. Whereas N-7 methylation is essential for RNA translation and stability the function of 2'-O methylation and its role in virus infection remained uncertain since its discovery 35 years ago until recently. Studies by members of our group have shown that 2'-O MTase activity of flaviviruses, coronaviruses, and poxviruses promotes viral evasion of Ifit family of genes, a group of IFN-stimulated innate immune effector proteins. Viruses lacking 2'-*O* MTase activity were attenuated in wild type primary cells and immunocompetent animals but were rescued in cells and mice lacking *Ifit1* gene expression. This data is consistent with a model in which 2'-O methylation of the 5' cap of viral RNA subverts innate host antiviral responses through escape of IFIT-mediated suppression, and suggest an evolutionary explanation for 2'-O methylation of cellular mRNA: to distinguish self from non-self RNA. The fact that cytoplasmic viruses cannot use nuclear host 2'O MTases and therefore encode their own viral 2'-O MTases attests to their evolutionary success against their hosts. Nonetheless, given that host 2'-O methylation of cellular mRNA largely occurs in the nucleus, pharmacological strategies that specifically disrupt cytoplasmic viral 2'-O MTase activity could represent a novel class of broad-spectrum antiviral therapy against a number of globally relevant human pathogenic viruses that replicate exclusively in the cytoplasm, including flaviviruses.

B. Goal. Identification of compounds that inhibit viral 2'-O MTase activity and sensitize flaviviruses to the antiviral effects of Ifit1. Compounds that inhibit WNV infection in Ifit1-expressing cells will be tested across a full dose-range for their activity in T-antigen transformed MEFs that ectopically express Ifit1. Small molecules that specifically block 2'-O MTase activity should have little or no inhibitory effect in *Ifit1*$^{-/-}$ cells but should function specifically in isogenic cells expressing Ifit1. Compounds that show this dependence on Ifit gene expression for inhibition of viral replication will be further analyzed as potential inhibitors of viral 2'-O MTase activity. Again, these 'hits' should have no effect on WNV-NS5-E218A, which already lacks 2'-O MTase activity. As final proof of their mechanism of action, lead compounds that sensitize flaviviruses to the effects of Ifit1 in cell culture will be tested *in vivo* for their ability to differentially inhibit flaviviruses in *Ifit1*$^{+/+}$ and *Ifit1*$^{-/-}$ mice.

C. Progress. Generation of inducible Ifit1-expressing MEFs.

1. Derivation of SV2-transformed MEFs. Murine embryonic fibroblasts (MEFs) were derived from 15 day-old *Ifit1*$^{-/-}$ C57BL/6 embryos and cultured *in vitro* for three passages. T-150 flasks of confluent MEFs were transfected with the pSV2 plasmid encoding the SV40 polyoma virus large T antigen. Transfected cultures were incubated at 37^{o}C and monitored for cell death with media being replaced ever three days to remove cellular debris. When colonies of transformed cells became visible (roughly 2 weeks), the cells were replated and then passaged ten additional times before being frozen in liquid nitrogen.

2. Production of FKBP-mIFIT1 inducible MEF line. SV2-immortalized Ifit1$^{-/-}$ MEFs (p20) were transduced with the lentiviral vector pMD144-FKBP-mIFIT1 (kind gift from H. Malik) in 6-well plates. The FKBP de-stabilization domain (DD) is appended to the N-terminus of IFIT1 and results in rapid degradation when expressed in mammalian cells; this allows transfection of the IFIT1 construct with low basal levels of expression in

Ifit1$^{-/-}$ MEFs. However, The FKBP-derived destabilizing domains are blocked by the addition of small molecules (e.g. Shield1); inclusion of Shield1 ligand in the medium stabilizes a DD-tagged protein of interest in a predictable and dose-dependent manner.

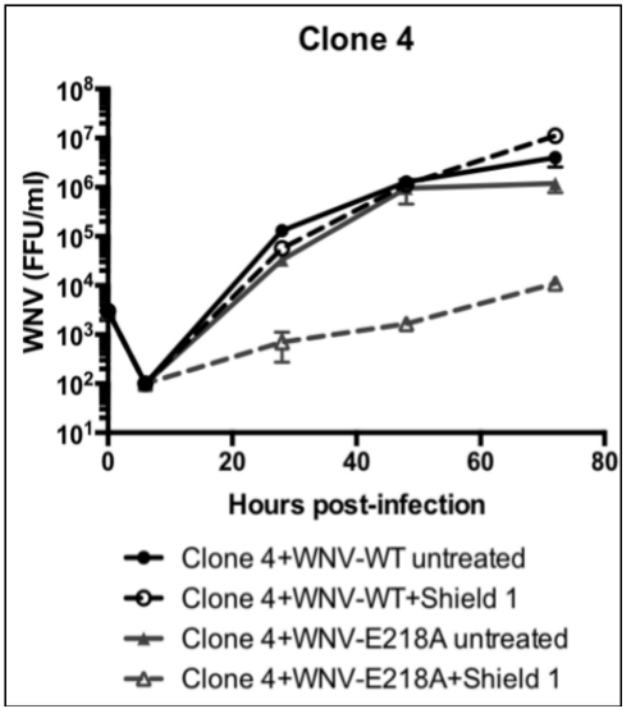

Figure 1. Induction of IFIT1 in Ifit1$^{-/-}$ MEFs using Shield1 ligand results in selective inhibition of WNV strains lacking 2'-O methylation (WNV-NS5-E218A). At baseline, ectopically expressed IFIT1-DD is rapidly degraded. Addition of Shield1 stabilizes expression of IFIT1 and confers an antiviral effect against WNV-NS5-E218A.

To generate this inducibe cell line, cells were transduced with the lentivirus and cells were selected with puromycin. After three days of drug selection, the culture media was replaced and the concentration of puromycin was reduced to maintain FKBP-mIFIT1 gene expression. Individual colonies were then picked and transferred to 6-well plates for expansion. Individual clones were then assessed for FKBP-mIFIT1 expression by flow cytometry (using an anti-IFIT1 monoclonal antibody) and mIFIT1 function by assessing replication of WNV-WT and WNV-NS5-E218A in the presence and absence of Shield-1 treatment.

In the absence of Shield1, Ifit1 expression was low and no difference in viral infection was observed in viruses having (WNV-WT) or lacking (WNV-NS5-E218A) 2'-O methylation (**Fig 1)**. In the presence of Shield1, Ifit1 expression was rapidly increased, and only viruses lacking 2'-O methylation became sensitized to the antiviral effects of Ifit1. Thus, an inducible cell line was generated and sent to SRI for subsequent HTS.

2. Production of doxycycline-inducible 293T cell line expressing mouse or human IFIT1. As an independent cell for high-throughput screening and validation, we have generated a 293T cells that expresses IFIT1 genes under a doxycycline inducible promoter. At baseline, IFIT1 is not expressed but with the addition of 1 μg/ml of doxycycline, IFIT1 is expressed as judged by flow cytometry (**Fig 2, left**). This level of IFIT1

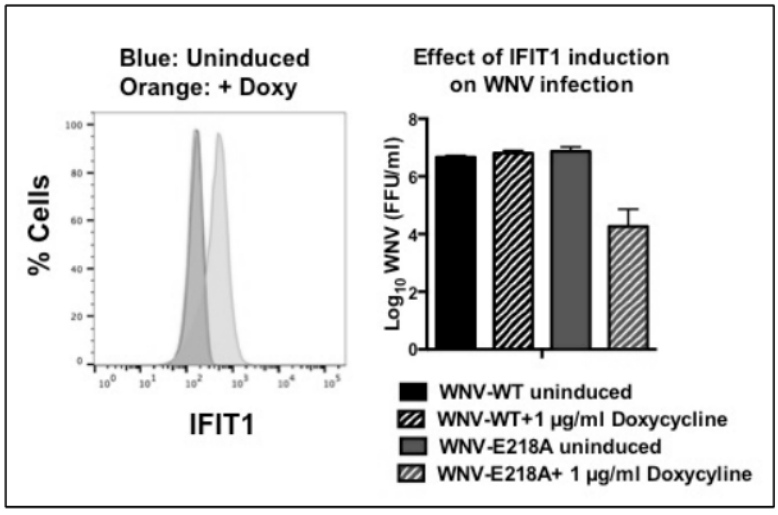

Figure 2. Induction of IFIT1 in 293T cells with doxycycline. Left. Flow cytometry histograms. Right. Viral yield assay at 48 h

expression is sufficient to inhibit infection of WNV strains lacking 2'-O methylation (WNV-NS5-E218A) but does not inhibit wild-type parent viruses (**Fig 2, right**). These cells have been shipped to Southern Research Institute for further high throughput screening.

3. Validation of hits from primary screen. A high-throughput screen recently was performed at SRI with untreated (no expression of IFIT1) and doxycycline (+ expression of IFIT1) treated 293T cells to identify compounds that selectively inhibit WNV-WT infection only when IFIT1 was induced. These compounds were recently shipped to the Diamond laboratory. Our plan will be to initiate EC50 and CC50 analysis in the 293-IFIT1 cells and confirm a lack of inhibitory activity in 293-only cells. Once completed, we will repeat EC50 and CC50 analysis in with WNV-NS5-E218A, which lacks 2'-O methylation due a point mutation in the NS5 methyltransferase. Candidate 'hits' should not inhibit WNV-NS5-E218A if they are acting to inhibit 2'-O methylation. Finally, as we now have ZIKV strains lacking 2'-O methylation (ZIKV-NS5-E217A), 'hits' will subsequently tested against another relevant falvivirus.

B.4. Training Opportunities.

The Office of Postdoctoral Affairs (OPA) encourages all postdocs to complete an individual development plan, and recommends using the tool myIDP hosted by Science Careers. New postdocs are introduced to IDPs and the myIDP tool during orientation and workshops are offered throughout the year. OPA recommends that faculty review Individual Development Plans with postdocs at their annual review. IDPs should be reviewed and updated at least annually.

We recognize that postdocs need both information and opportunities to explore the variety of career outcomes pursued by our alumni. OPA has an Education Coordinator and the University employs a full-time career strategist to provide career and professional development training along with Career Talks for postdocs.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	We have generated the doxycycline inducible 293T cell that expresses IFIT1. Upon publication, we will deposit this cell line at BEI Resources (ATCC) for use by the greater scientific community.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Michael		Diamond		Project Leader	(b)(4); (b)(6)				9,255.00	1,920.00	11,175.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,175.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,486.00	4,241.00	19,727.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Sr. Scientist, Sr. Research Technician				38,857.00	11,506.00	50,363.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**70,090.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**81,265.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	69,596.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Equipment Service Contracts	2,500.00
9. Machine Shop/Computer Maintenance	2,000.00
10. Glassware/BSL3 Waste Disposal	3,000.00
Total Other Direct Costs	**79,096.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**163,361.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC on campus	52.5	163,361.00	85,765.00
		Total Indirect Costs	**85,765.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX 214-747-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**249,126.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Budget Justification Whitley Diamond-Year 4.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

PERSONNEL:

Michael S. Diamond, M.D., Ph.D., Co-Investigator. Professor of Medicine, Molecular Microbiology, Pathology and Immunology at Washington University School of Medicine. Dr. Diamond will devote (b)(4) months to this project and receive (b)(4) months of salary support. He will be responsible for the design and oversight of all investigations occurring in his laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

James White, Ph.D. Post-doctoral Research Associate. Dr. White is a post-doctoral associate that has been in the Diamond lab for more than 4 years. He has significant experience in flavivirus biology and will perform studies in cell culture to test the effects of lead hits on WNV and other viruses. He will devote ~(b)(4) months of time to this project and derive (b)(4) months salary from it. He will be responsible for interpreting data and troubleshooting technical problems in consultation with the principal investigator.

Jennifer Govero, PhD. Sr. Research Associate. Dr. Govero has significant experience (~7 years post-Ph.D.) in virology, immunology, and animal models of disease. She will devote (b)(4) months of her time to this project and derive (b)(4) months of her salary accordingly, beginning in FY2. One of her primary roles will be participating in the testing of small molecules in mice. She also will work with Dr. Austin on some of the virological studies in characterizing the lead molecules in vitro and in vivo.

Michelle Noll, Animal Technician. This project requires a significant amount of animal work associated with breeding of Ifit1 KO mice, genotyping, and conducting animal experiments. Ms. Noll, our experienced animal technician, will be responsible for animal husbandry under the oversight of Drs. Govero and Diamond. She will devote (b)(4) months to the project and receive (b)(4) months of salary support in FY 3-5.

EQUIPMENT: No new equipment is needed.

SUPPLIES:

Tissue culture ($7,000). With this project, there will be a considerable amount of tissue culture associated with cell-based validation of small molecule hits and target gene identification. The funds requested will be used for media preparation, growth additives, primary cell culture, serum, antibiotics, plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles).

Molecular Biology Reagents ($7,500). This amount has been budgeted for reagents for molecular cloning (restriction enzymes), proteases, transfection (liposomes, electroporation cuvettes), plasmid DNA and RNA purification kits, electroporation, vectors, bacterial culture supplies, and DNA/RNA/protein electrophoresis. .

Chemicals ($2500). This amount has been budgeted for general chemical supplies including buffers, salts, organic solvents, acids, bases, and detergents.

Immunochemical and Reagents ($3,500). This has been budgeted for the direct labeling of antibodies with different fluorophores, for secondary reagents for ELISA, and for intracellular immunofluorescence studies. Some validation screening in different cell types will be performed using immunofluorescence as the readout. This budget also includes time on a shared confocal microscope (~$100/hour).

Real-time RT-PCR ($5,000). We have developed a sensitive and reproducible quantitative real-time RT-PCR for assessing flavivirus replication using an ABI 7000 Sequence Detection instrument. This assay has become our standard for RNA quantitation and will be used to measure viral RNA levels in serum and in tissue and cells. RT-PCR reagents, primers, Taq-Man probes cost approximately $1 per well. In addition, for most samples, a ribosomal or actin RNA control is run for normalization purposes. Also, some of the in vivo studies will use RNA-based methods for quantitation.

Flow Cytometry Reagents and equipment time ($2,500). This amount is for reagents associated with running of the flow cytometer including calibration, buffers, controls, and software licenses. The flow cytometry will be used for screening and validation small molecules that restruct WNV infection.

DNA Oligonucleotides ($1,000). We will use the supplier of the core facility at Washington University which charges approximately $0.20 per base. These oligonucleotides will be used for cloning and sequencing.

Liquid N2/CO2 ($1,000). This amount is budgeted for liquid N2/CO2 bottled gas and related tank rental fees.

Animal pharmacy/sentinal testing/dissection ($1,000 – *FY3-5*). This reflects the costs of anaesthetics and surgical tools for necropsy, and the charges for sentinel testing.

Animal Puchases, Breeding, and Housing ($37,596 – FY3-5). Beginning in FY3, the costs reflect the purchase and breeding for all prophylaxis and therapeutic experiments with small molecules. Also built into these costs are the purchase of Alzet osmotic pumps for drug delivery.

OTHER EXPENSES:

Equipment Service Contracts ($2,500). This money is budgeted for service contracts on all essential equipment to be used with this grant ELISPOT reader (for viral focus forming assays), our 96-well plate flow cytometer, and ABI 7500 TaqMan machine.

Other ($5,000). This money is budgeted for additional costs associated with operation (disposable gowns, gloves, boots, barrier tips) and usage of the BSL3 facilities along with waste disposal (3,000). Funds are also budgeted for machine shop and computer maintenance ($2,500).

Publications ($2,000) we request support on publishing at least one manuscript per year ($2,000).

Travel ($3,000) Travel has been budgeted for one reverse site visit with NIH, one meeting with the Whitley/Nelson laboratories, and a scientific meeting to present data.

A. COMPONENT COVER PAGE

Project Title: Project 2.2 Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics
Component Project Lead Information: Baric, Ralph S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of Project 2 is to identify inhibitors of two highly conserved CoV processes, replication fidelity and RNA capping, that are essential for SARS-CoV virulence and survival in vivo. Multiple viral proteins and enzymatic activities are critical for these processes, including CoV 3'-to-5' exoribonuclease (fidelity; nsp14-ExoN) and 2'-O-methyltransferase (capping; nsp16-OMTase) activities. Consistent with the importance of these processes, we have shown that decreased replication fidelity and ablation of RNA capping through genetic inactivation of either ExoN or OMTase, respectively, results in replication competent viruses that are profoundly attenuated in vivo.

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication. We will test the hypothesis that inhibitors of CoV high-fidelity replication will decrease viral fitness alone and in combination with RNA mutagens, and represent potent pan-CoV therapeutics. In part 1, we will identify ribonucleoside analogs that inhibit CoV replication, and define their mechanism of action. High-throughput screening in part 2 will identify small-molecule inhibitors of CoV fidelity. In part 3 we will identify the viral protein targets of lead compounds, and determine their mechanism of fidelity impairment. In part 4, will we test highly efficacious compounds identified in parts 1 and 2 across the CoV family and viral platforms within this program.

Aim 2. To identify and develop inhibitors of CoV RNA capping activity. We hypothesize that small molecule inhibitors of essential CoV RNA capping components will profoundly increase CoV sensitivity to the host innate immune response through interferon-stimulated effectors. In part 1 we will use targeted mutagenesis of known CoV capping components to define distinct mechanisms to increase CoV sensitivity to the host ISGs. In part 2 we will examine the combined efficacy of known O-MTase inhibitors and type I IFN treatment against SARS-CoV, and perform a high-throughput screen for inhibitors of CoV RNA capping. In part 3 we will identify the viral protein targets and mechanism of action of lead compounds. In part 4, lead compounds will be tested across the CoV family and specific viral platforms within this program.

Aim 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors. We will test the hypothesis that inhibitors of CoV fidelity or RNA capping are highly attenuating in vivo and represent broadly effective CoV therapeutics. Compounds identified in Aims 1 and 2 will be chemically optimized for in vitro efficacy, selectivity, solubility, microsomal stability, and bioavailability at SR. Using these optimized compounds, in part 1 we will confirm the biological target(s) of lead fidelity and RNA capping inhibitors in vivo. In part 2 we will test the efficacy of lead compounds against mouse-adapted SARS-CoV in progressively stringent mouse models of acute and persistent human disease. Efficacy will be determined by monitoring respiratory function, morbidity and mortality, histology, and viral replication. In part 3 we will test for the development of drug resistance in vivo, and will determine the efficacy of lead compounds against MERS-CoV and other CoV family members.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Project 2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for the next reporting period are based on continuation of the sections above. Briefly, hits from the SRI HTS will be assessed in vitro with MHV followed by SARS and MERS and if promising, compounds will be evaluating for efficacy within in vivo models of CoV pathogenesis. The continued preclinical development of GS-5734 will be the main focus of both (b)(6); and Baric labs. Given that the resistance mutations generated thus far only provide a moderate shift in EC50 (5-fold), the (b)(6); (b)(3):7 U.S.C. § 8401 will continue to select for mutations that further enhance resistance for both SARS- and MERS-CoV. In addition, the will lead efforts to determine the mechanism of action for GS-5734. The Baric lab will continue to assess GS-5734 efficacy in primary human cells that drive SARS- and MERS-CoV pathogenesis. In additional focus will be in vivo efficacy studies with genetically divergent bat CoV and MERS-CoV in newly developed transgenic mouse models. Our work on GS-5734 thus far will serve as key data for the preclinical package to be submitted by Gilead for IND licensure in the last quarter of 2016.

1.Preclinical development of GS-5734 in partnership with Gilead (b)(6); (b)(3):7 U.S.C. § 8401 and Baric Labs.

(b)(6); (b)(3):7 U.S.C. § 8401 ab

•Force MHV adaptation to increasing concentrations of GS-5734 or its parent compound to identify the maximum achievable level of resistance and associated additional mutations in the RdRp or other replicase proteins, thereby further revealing GS-5734 mechanism of action and CoV pathways to resistance.
•Select, genotypically analyze, and phenotypically characterize GS-5734-resistant MERS-CoV and SARS-CoV mutant viruses in Calu-3 cells. Evaluate impact of GS-5734 resistance mutations on replicative fitness of MHV, SARS-CoV, and MERS-CoV in cell-culture.
•Systematically examine steps and processes in the CoV life cycle affected by GS-5734 using wild-type, GS-5734-resistant, and RNA proofreading-impaired viruses.
•Select, genotypically analyze, and phenotypically characterize EIDD-1931-resistant MHV, MERS-CoV, and SARS-CoV mutant viruses. Determine the nature of functional interactions—synergistic, additive, indifferent, or antagonistic—between GS-5734 and EIDD-1931 when combined against wild-type, GS-5734-resistant, or EIDD-1931-resistant MHV, MERS-CoV, and SARS-CoV.
•Determine sensitivity of GS-5734-resistant mutant viruses to other classes of antiviral compounds, including mutagens (e.g., 5-fluorouracil, ribavirin, and 5-azacytidine) and inhibitors of RNA polymerization (e.g., 2' C-methyl adenosine).

Baric Lab

•Evaluate antiviral efficacy of SARS- and MERS-CoV in various primary human cells that guide in vivo pathogenesis. Antiviral assays in primary human type II pneumocytes, lung fibroblasts and endothelial cells, etc.
•Perform EC50/EC90 studies in HAE with parent/prodrug GS compounds that were effective at 1uM or less in previous pilot studies.
•In vivo efficacy studies with MERS-CoV in newly generated transgenic mice (Ces1c-/-/288/330+/+).
•In vivo efficacy studies with SARS-like bat CoV (HKU3) and MERS-like bat CoV (HKU5).
•Evaluation of in vivo pathogenesis of SARS- and MERS-CoV resistance mutants and assess their ability to counteract treatment with GS-5734 in mice.

2.Evaluation of hits from SRI HTS in partnership with SRI, UAB (b)(6); (b)(3):7 U.S.C. § 8401 nd Baric Labs.

•Confirmation of antiviral activity of hits from HTS with MHV, SARS- and MERS-CoV (b)(6); (b)(3):7 U.S.C. § 8401
•Build SAR for additional medicinal chemistry efforts on current leads (SRI (b)(6); (b)(3):7
•For lead candidates, initiate passage for resistance, deep sequencing and possible mechanism (b)(6); (b)(3):7 U.S.C. § 8401
•Testing in HAE cells of verified active compounds (Baric).
•Establish possible candidates for in vivo (mouse model) testing (Baric)

Project 2: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics (b)(6); (b)(3):7 U.S.C. § 8401 Ralph S. Baric)

B.2. What was accomplished under these goals?

B.2.a. Major activities, specific objectives, significant results and key outcomes. Our collaborations with Southern Research Institute (SRI) and Gilead Sciences (GS) continue to generate significant and exciting results. Our major activities within the past reporting period include: 1) completion of a high-throughput screen (HTS) at SRI identifying two lead compounds 2) determination that Gilead compound GS-5734 is broad-spectrum with family-wide activity against CoV in human primary airway epithelial (HAE) cell cultures 3) completion of extensive metabolic and pharmacokinetic profiling of GS-5734 in collaboration with GS and demonstration that GS-5734 administered prophylactically or therapeutically improves lung function, lowers virus lung titers, and protects against weight loss and 4) determination that transfer of resistances mutations identified in mouse hepatitis virus (MHV), V553L and F476L, to SARS-CoV transfers the resistance phenotype and that these resistance mutations do not attenuate SARS-CoV pathogenesis in mice and 5) preparation of related grants and manuscripts.

1. SRI HTS reveals hits against SARS-CoV. The main objective of our collaboration with SRI was to perform an unbiased high-throughput screen (HTS) to identify compounds with antiviral activity against SARS-CoV. The initial CPE based HTS of >200K compounds identified 6 compounds with robust antiviral activity that were confirmed with completely separate batches of compound. Medicinal chemistry was performed at SRI to optimize the chemical properties (solubility, microsomal stability, etc.) while retaining antiviral activity. These efforts yielded two compounds, 33911 and 36565, with acceptable EC_{50} values while only one had decent solubility (36565). In the coming year, the antiviral activity of 36565 will first be confirmed and validated in vitro with model CoV (mouse hepatitis virus, MHV) and then evaluated with both SARS- and MERS-CoV in a human lung epithelial cell line (i.e. Calu-3 2B4) and if successful in primary human airway epithelial cells (HAE). If proven to be robustly antiviral against SARS- and MERS-CoV in vitro, SRI molecules will be evaluated in vivo in mouse models of CoV pathogenesis.

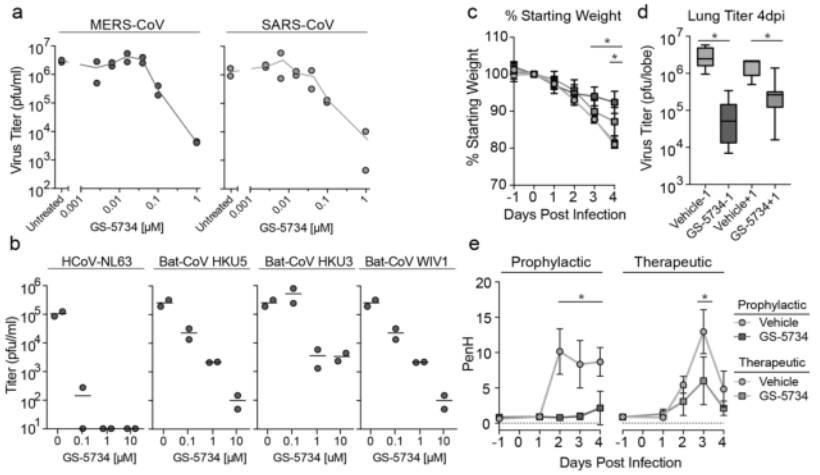

Figure 1: Antiviral efficacy of GS-5734 in primary human airway epithelial (HAE) cell cultures and mice. A) MERS- and SARS-CoV-infected HAE (MOI = 0.5) treated with increasing doses of GS-5734. B) HAE treated and infected as in Panel A. Group 1 human CoV NL63, group 2C MERS-like bat CoV HKU5, divergent group 2b bat CoV HKU3, and SARS-like prepandemic bat CoVs WIV1. Vehicle or GS-5734 (25 mg/kg) was administered twice daily beginning either day -1 or day +1 post-infection. C) Percent of starting weight demonstrating protection from weight loss with GS-5734 treatment. D) SARS-CoV titer in the lung is reduced with GS-5734 treatment. E) Pulmonary function as measured by whole-body plethysmography. Penh is a measure of airway obstruction.

2. GS-5734 is a broad-spectrum antiviral against CoV (*Baric Lab*; (b)(6); (b)(3):7 U.S.C. § 8401 *Sheahan*. (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 The main objective of our collaboration with Gilead has been to accelerate the preclinical evaluation of antiviral compounds against CoV. We have evaluated almost 30 different parent or prodrug nucleoside analogs for antiviral activity against SARS- and MERS-CoV in HAE with the parent GS-441524 and prodrug GS-5734 being the most promising. GS-5734, currently in clinical development (Phase II trials) for treatment of Ebola virus disease, inhibits SARS-CoV and MERS-CoV replication in multiple in vitro systems including HAEs with submicromolar EC_{50} values (SARS- and MERS-CoV EC_{50} = 30nm, **Fig. 1a**). GS-5734 was also effective against bat-CoVs, prepandemic bat-CoVs and circulating contemporary human CoV in primary human lung epithelial cells, thus demonstrating broad-spectrum anti-CoV activity (**Fig. 1b**). These data demonstrate that the prodrug, GS-5734, is efficiently transported and metabolized by key target cells of both SARS- and MERS-CoV in vivo. To ensure that phenotypes observed in primary human cells were not donor-specific, compounds are evaluated in cells from at least three donors. Since both SARS- and MERS-CoV target multiple cell types in the lung, future efforts will focus on determining antiviral efficacy in key primary cell types that mediate in vivo pathogenesis (i.e. type II pneumocytes, lung fibroblasts and endothelial cells, etc.).

3. Both prophylactic and therapeutic GS-5734 protects mice from SARS-CoV induced disease *(Gilead and Baric Lab, Sheahan).* To accelerate the preclinical development GS-5734, in collaboration with GS, we have performed extensive in vitro metabolism and in vivo pharmacokinetic (PK) analysis. GS-5734 has relatively poor plasma stability in mice due to expression of a secreted carboxylesterase 1c (*Ces1c*) absent in humans and non-human primates. Thus, all PK and antiviral efficacy studies were performed in *Ces1c*$^{-/-}$ mice. These studies demonstrated that drug was metabolized to the active triphosphate (TP) in the lungs of mice administered GS-5734 subcutaneously and that a twice daily dosing (BID) regimen was able to maintain trough levels consistent with those anticipated in humans and sufficient to maintain CoV inhibition over the dosing interval (data not shown). Importantly, with both prophylactic (i.e. dosing beginning 1 day before infection) and therapeutic (i.e. 1 day post infection) BID dosing via the subcutaneous route, we demonstrated antiviral efficacy against SARS-CoV with significant reductions in titer, improved lung function and minimal weight loss with treatment as compared to vehicle treated controls (**Fig. 1c-d**). The observed antiviral effect was independent of sex and age. Both SARS- and MERS-CoV pathogenesis is increased with increasing age in humans and this phenotype can be modeled in adult and aged mice. Thus, protection of both adult (20-28 week old, Fig. 1c-d) and aged (60+ week old, data not shown) suggests that GS-5734 treatment in even the most vulnerable human populations may be effective. Since the murine ortholog of the human MERS-CoV receptor (DPP4) does not support MERS-CoV infection, in collaboration with Gilead, we are generating a mouse deficient in *Ces1c*$^{-/-}$ containing human DPP4 alleles at positions 288 and 330 (*Ces1c*$^{-/-}$/288/330$^{+/+}$) to facilitate in vivo efficacy studies with MERS-CoV. In addition, we have additional mouse models for genetically divergent bat CoV (HKU3 and HKU5) within which we will assess the breadth of efficacy in vivo.

4. Resistance phenotypes are genetically transferable among CoV (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 *Baric Lab,* (b)(6); (b)(3):7 U.S.C. § 8401 *Sheahan)* MHV serves as a highly informative, tractable, and efficient BSL-2 model system for studies of CoV genetics, replication mechanisms, and viral evolution. The (b)(6); (b)(3):7 U.S.C. § 8401 Lab has shown that MHV can be used to rapidly screen compounds for potentially broad activity against divergent CoV's. Through selection and genotypic and phenotypic analysis of MHV isolates resistant to antiviral compounds, the (b)(6); (b)(3):7 U.S.C. § 8401 ab has obtained insights into mechanisms of action and genetic barriers to resistance. Of crucial importance, mutations in the MHV RdRp that confer resistance to GS-5734—V553L and F476L—recapitulate the resistance phenotype when introduced into SARS-CoV. As amino acid residues at these positions are absolutely conserved across diverse CoV's, we fully expect that MERS-CoV and other human and bat CoV's engineered with the V553L and F476L substitutions will phenocopy MHV resistance to GS-5734. We further anticipate that MHV will usefully serve to screen other lead compounds for anti-CoV activity, elucidate viral targets of these agents, identify potential pathways leading to resistance, and illuminate means of preventing or therapeutically circumventing selection of drug-resistant variants. To the latter point, the (b)(6); (b)(3):7 U.S.C. § 8401 Lab recently made the exciting observation that V553L and F476L mutations, while fostering resistance to GS-5734, actually enhance MHV susceptibility to a cytidine analog developed at the Emory Institute for Drug Discovery, EIDD-1931. This complementary activity relationship between GS-5734 and EIDD-1931 demonstrates the potential to treat CoV infections with drug combinations that prevent emergence of clinical resistance. The (b)(6); (b)(3):7 U.S.C. § 8401 Lab will continue using MHV to initially evaluate the impact of resistance mutations on the efficacy and potency of structurally similar and dissimilar compounds with anti-CoV activity, followed by testing of promising drug combinations against human CoVs.

5. Preparation of related grants and manuscripts. To accelerate the preclinical development of GS-5734 for the MERS-CoV indication and IND licensure, we have applied for a Partnerships for Countermeasures Against Select Pathogens (R01, RFA-AI-16-034) grant. We have also submitted a publication detailing our in vitro and in vivo evaluation of GS-5734 titled "Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses" to Science Translational Medicine. An additional manuscript describing resistance to GS-5734 is in preparation.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Ralph		Baric		Project P.I.	(b)(4); (b)(6)				12,957.00	3,361.00	16,318.00
2.	Dr	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					23,554.00	6,691.00	30,245.00
3.	Dr	Timothy		Sheahan		Co-Investigator					19,615.00	5,790.00	25,405.00
4.	Dr	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					21,321.00	6,294.00	27,615.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **99,583.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
		(b)(4)					
2	Post Doctoral Associates				21,857.00	3,904.00	25,761.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Specialist				14,636.00	4,481.00	19,117.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**44,878.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**144,461.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	115,489.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Deep Sequencing; Maintenance Contracts	15,000.00
9. Histology; Flow cytometry	13,000.00
10. Animal per diem; shipping	8,421.00
Total Other Direct Costs	**153,910.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**304,371.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.0	304,371.00	158,273.00
		Total Indirect Costs	**158,273.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**462,644.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Year_4_Budget_Justification_Whitley CETR Baric.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification Baric Project Whitley CETR Proposal Year 4

Personnel:

Ralph Baric, Ph.D., Principal Investigator ((b)(6) , (b)(6) months). Dr. Baric will supervise the overall direction of the animal research agenda of this highly interactive proposal. He will interact closely with Drs. (b)(6); (b)(3):7 U.S.C. § 8401 nd Sheahan, and (b)(6); (b)(3):7 U.S.C. § 8401 to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. Dr. Baric will be also be responsible for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with th (b)(6); (b)(3):7 U.S.C. § 8401 laboratory, writing progress reports and managing fiscal matters associated with the proposal. Given the extensive interaction and collaboration with Dr. (b)(6); (b)(3):7 U.S.C. § 8401 in the past, he will also lead efforts to coordinate and promote research efforts with the groups. Dr. Baric will communicate his findings with D (b)(6); (b)(3):7 U.S.C. § 8401 on a regular basis via both conference calls and meetings between the two laboratories.

(b)(6); (b)(3):7 U.S.C. § 8401 **.D. Co-Investigator** (b)(4) months). (b)(6); (b)(3):7 U.S.C. § 8401 will oversee all select agent research in the facility (b)(6); (b)(3):7 will test select SARS-CoV and MERS-CoV mutants in primary culture models to evaluate drug candidates in the more advanced human in vitro model. In addition (b)(6); (b)(3):7 will perform viral passage studies in the presence of drug candidates to identify escape mutations that may arise (b)(6); (b)(3):7 U.S.C. § will report findings regularly to Drs. Baric and (b)(6); (b)(3):7 U.S.C. § 8401 as well as interfacing with the drug candidate manufacturers.

Timothy Sheahan, Ph.D. Investigator (b)(4) months). Dr. Sheahan has extensive BSL3 experience and has recently rejoined the Baric laboratory as a Research Assistant Professor. He will lead the in vivo drug testing with wild type virus portion of the project in collaboration with Dr (b)(6); (b)(3):7 U.S.C. § 8401 as well as performing many of the in vivo experiments. He will be assisted by Drs. (b)(6); (b)(3):7 U.S.C. § 8401 and (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401 **Investigator** (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 will lead the in vivo testing of compounds with SARS-ExoN I experiments and the in vivo virus evolution experiments proposed in the application (b)(6); (b)(3):7 in collaboration with Dr. Sheahan will lead our efforts in studying the pathogenesis of SARS-CoV ExoN mutants, derivative ExoN evolved viruses, and conducting in vivo persistent infections/evolution experiments in animals. (b)(6); (b)(3):7 has extensive experience working with ExoN I in mice and is skilled at assembling recombinant SARS-CoV viruses using classic recombinant DNA approaches or synthetic genomic approaches. (b)(6); (b)(3):7 U.S.C. § will work closely with Drs. Baric (b)(6); (b)(3):7 and Sheahan for experimental design and analysis.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months (b)(6); (b)(3):7 U.S.C. § 8401 in the Baric laboratory who is learning to work with our infectious clone platform and is interested in virus infection of primary human cell types (b)(6); (b)(3):7 U.S.C. § 8401 will work with (b)(6); (b)(3):7 U.S.C. § 8401 to analyze the drug studies in primary human lung cells and will report all findings to Drs. Baric, Sheahan and (b)(6); (b)(3):7 U.S.C. §

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 in the Baric laboratory who is training now to independently enter the Baric BSL3 laboratory. (b)(6); (b)(3):7 U.S.C. § 8401 has extensive experience infecting mice with human influenza virus and will be working with Drs. Sheahan and (b)(6); (b)(3):7 U.S.C. § 8401 to perform the animal infections, assay samples and to analyze data. (b)(6); (b)(3):7 will communicate all findings to Drs. Baric, Sheahan an (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401)(4) months (b)(6); (b)(3):7 U.S.C. § 8401 has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal husbandry. (b)(6); (b)(3):7 will also be responsible for purchasing supplies, maintaining stocks in the BSL3 and will support Drs. (b)(6); (b)(3):7 U.S.C. § 8401 and Sheahan's research efforts as needed.

Fringe Benefits: Faculty/Staff: 22.883% Social Security and Retirement; $5,659/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,310/FTE Health Insurance.

Travel

Travel: ($6,000): Domestic Travel: Funds are requested for the Project Leader and staff to attend 2 scientific conferences and the annual CETR U19 meeting in Bethesda each year. This allows program faculty and fellows to communicate results, develop collaborations and share research interests.

Supplies:

Molecular Biology Reagents ($7,000/year) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic acid markers. As sequence confirmation is critical prior to assembly of full length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of ExoNI mediated mutations.

Synthetic DNA ($5,500/year) Funds are requested for the purchase of synthetic DNA fragments which are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc. at costs of about $0.35/base. Our budget allows for ~40,000-bp of synthetic gene synthesis/yr, sufficient for our needs over the course of this project and will allow for the rapid assembly of recombinant viruses bearing different ExoNI derived mutations.

BL3 Protective Gear ($10,000/year) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Miscellaneous ($5,989/year) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings.

Computer Supplies ($1,000/year) Funds are requested for project specific computer and software upgrades over the course of the proposal.

Tissue culture ($40,000/year) Funds are requested to purchase mature human airway epithelial cell (HAE) cultures for drug testing assays. Each culture is $100. We anticipate requiring 300 HAE cultures. In addition, we are requesting funds to purchase cell culture supplies and plasticware to perform virus plaque assays and general tissue culture work.

Immunology Reagents ($6,000/year) Funds to purchase supplies for flow cytometry and ELISA based immune assays are requested to cover the cost of purchasing fluorescently tagged antibodies for the purposes of immune-phenotyping inflammatory cells.

Animals ($40,000/year) Funds are requested to purchase ~160 each- SCID ($68), RAG ($124), young BalbC ($25), aged BalbC ($20), young B6 ($20) and aged B6 ($18) mice at the indicated prices per mouse. In addition, funds are requested to purchase ~60 golden Syrian hamsters ($43). These monies are essential for evaluating drug efficacy across hosts of differing susceptibilities to lethal infection, and to test drug efficacy in at least two animal species.

Other Expenses:

Animal per diem ($7,800/year) SCID, RAG and the young BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~30 days prior to the start of experiments (5 animals per cage x 30 days x 0.65 per cage). The aged BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~90 days prior to the start of experiments (5 animals per cage x 90 days x 0.65 per cage).

Deep Sequencing ($10,000/year) The ExoN mutator phenotype results in high mutation rates which must be accessed by ultra deep sequencing methods like RNAseq, including informatics support to analyze the data. This also includes funds for supplies to generate amplicon library and to prepare the library for sequencing. As such, we anticipate significant sequencing costs over the duration of this proposal.

Maintenance Contracts ($5,000/year) Several instruments in the Baric Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes, BSL3 autoclaves) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology ($5,000/year) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Flow Cytometry ($8,000/year) UNC-Chapel Hill provides a core facility with advanced analytical cytometers that can resolve >6 colors at a time, which is needed when delineating subsets of inflammatory cells following infection.

Publication costs ($2,000/year) Funds are requested to cover the publication of manuscripts.

Shipping ($621/year) Funds are requested to cover the costs of shipping samples/viruses to the laboratory for analysis over the course of the proposal.

(b)(6); (b)(3):7 U.S.C. § 8401

A. COMPONENT COVER PAGE

Project Title: Project 3.2 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

(b)(6); (b)(3):7 U.S.C. § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, “hits” will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50 , CC50 , and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds.
Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 Project 3.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

1.Quinolones (SR-33394): Identify the mechanism of action for this class of compounds using in vitro assays. These will be tested for activity. Colorado will characterize VEEVTC83 virus that displays resistance to SR-34329 by utilizing sequence information generated by UNC to introduce the mutations into the infectious clone for VEEVTC83. SR plans to synthesize a new series based upon current SAR. Further SAR and testing will be dependent upon the results of the new analog series.
2.Tetralins-Benzo Annulenes (SR-33366): Additional SAR for this chemical series will be completed in order to optimize activity with stability and bioavailability. We plan to finish mechanism of action (MOA) studies for this compound series utilizing resistance phenotype information, in vitro assays and protein/compound binding. Since this compound series displays activity against a wide range of virus families; we will further characterize this property in order to determine the range of activity. In vivo pharmakokinetic (PK) analysis will be completed allowing us to determine dosing amount, route and timing for efficacy studies in our mouse models of CHIKV infection and disease.
3.VEEV 2015 HTS: SRI-36426 and SR-36427 were chosen for SAR due to their high activity and low toxicity profiles. SR will continue to provide the group with additional analog for these two scaffolds for SAR. PK analysis will be preformed on the most active compounds. Since SR-36426 is broadly active against 5 different Alphaviruses; we will determine the breadth of activity against other viruses. MOA studies should be completed for SRI-36426 and SR-36427.
4.CHIKV 2015 HTS: SR-36767 & SR36768 are the new leads for CHIKV but both compounds block a number of other Alphaviruses. We will continue to perform MOA and breadth of action studies for these two series. SR will continue to synthesize new analogs in order to optimize compound activity and stability. We hope to perform PK analysis and in vivo testing for these two series.
5.Project 1, 2, 4 Hits: We will continue to test additional compounds that are active against viruses from the other projects in order to identify broadly active compounds. We plan to determine the MOA for DENV compound SR-37014 because this was the most active compound identified in the cross-screens performed in 2016.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

Progress towards our goals is outlined for each Specific Aim:

SA1. HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screen: *VEEV HTS* identified 940 active samples and 8 out of 12 sent to OHSU had activity in NHDFs. *CHIKV HTS* identified 2,558 active compounds and 5 out of 11 were confirmed.
2. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified. OHSU tested 35 and found 4 actives against CHIKV. SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR and mode of action studies.
3. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication, Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). OHSU validated four anti-VEEV compounds as effective against CHIKV in these cells.
4. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate HTS and SAR including a new CHIKV strain expressing nano-Luciferase provided by UNC. Other recent isolates from Puerto Rico have been cloned and sequenced. These highly relevant strains may be used in subsequent validation experiments.
5. $VEEV_{TC83}$ has also been modified to encode nluc and is currently being validated at Colorado. $VEEV_{TC83}$-nLuc will be used by SR for SAR studies and the group for antiviral validation studies.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens and to identify the mode of action for the lead hits. To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has undertaken the optimization of specific assays that they will utilize to test lead compounds.
2. **Quinolones (SR-33394):** SR synthesized 89 analogs. OHSU tested them in virus reduction assays and found the active compounds SR-33394 (EC_{90}=0.77μM), SR-34329 (EC_{90}=0.12μM), SRI-36506 (EC_{90}=4.9μM) and SR-36959 (EC_{90}=0.78μM). All other analogs had decreased activity compared to SR-33394. Colorado generated a $VEEV_{TC83}$ virus that displays resistance to SR-34329, which is being sequenced by UNC.
3. **Tetralins-Benzo Annulenes (SR-33366):** SR synthesized >73 analogs of SR-33366 for SAR. SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC_{90}=0.45μM compared with SR-33366 (EC_{90}=3.2μM). Sequencing of UNC- and OHSU-derived resistance mutants identified changes in the macrodomain of NSP3. This finding is consistent with mode of action studies showing that SR-34963 blocks viral RNA synthesis at the level of subgenomic RNA synthesis. SR is performing crystallization/binding assays with NSP3. Analog SR-36429 (EC_{90}=1.5μM) showed better microsomal stability and PK activity and may be used for *in vivo* activity experiments. Additional recent analogs show activity and are under SAR. SR-34963 is also active against Flaviviruses (DENV, ZIKV), Coronaviruses, and Influenza virus.
4. **VEEV 2015 HTS:** OHSU confirmed 8 of 12 active hits including: SR-36415 (IC_{90}=0.77μM), SR-36416 (IC_{90}=0.35μM), SR-36420 (IC_{90}=0.13μM), SR-36421 (IC_{90}=0.11μM), SR-36423 (IC_{90}=0.22μM), SR-36424 (IC_{90}=0.06μM), SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM). SRI-36426 and 27 were chosen for further SAR. SR-36426 is active against 5 different Alphaviruses and blocks infection prior to viral RNA synthesis. SR-36427 is active against VEEV and Mayaro virus and blocks infection after RNA synthesis. Both work in $IRF3^{-/-}$ fibroblasts indicating that they do not function through IFN.
5. **CHIKV 2015 HTS:** OHSU confirmed 5 of 11 including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), SR-36767 (IC_{90}=0.09μM), and SR-36768 (IC_{90}=0.23μM). Two compounds (SRI-33001 and -36768) were active against 5 different Alphaviruses and SRI-36767 was active against 4 Alphaviruses. SR-36767 & -68 are the new leads for CHIKV and block infection prior to RNA synthesis. SR-33001 blocks viral replication at a step after viral RNA synthesis.
6. **Project 1, 2, 4 Hits:** DENV compound SR-37014 (IC_{90}=0.4μM) was active against CHIKV. SARS compounds SR-35742, -35894 and -36565 showed activity against VEEV but not CHIKV.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test *in vivo* efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease, including models of disseminated inflammation based on unique mouse strains from the Collaborative Cross Mouse Genetics Resource as well as the generation of a mouse-adapted CHIKV strain developed at Colorado that displays enhanced replication, dissemination, and pathogenicity in WT mice; 2) Intranasal inoculation of VEEV for neurological infections; 3) chronic CHIKV infection and joint disease in wild-type and immunodeficient mice; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of nonhuman primates. Each lab has unique models that they will use for testing lead compounds; this will increase our ability to quickly determine the *in vivo* efficacy profile for each lead.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6); (b)(3):7 U.S.C. § 8401			PhD	Consortium P.I.	(b)(4); (b)(6)				17,140.00	4,488.00	21,628.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,628.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	2 Senior Scientist, 1 Research Technician	(b)(4)			51,310.00	16,551.00	67,861.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**67,861.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**89,489.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	47,011.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Histology Core	5,000.00
9. Service Contracts and Maintenance	4,500.00
Total Other Direct Costs	**56,511.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**150,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.0	150,000.00	78,000.00
		Total Indirect Costs	**78,000.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**228,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: A3DC-UNC Justification 2016.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel

(b)(6); (b)(3):7 U.S.C. § 8401 Ph.D., Co-Investigator (b)(4) Months. (b)(4). (b)(6); (b)(3):7 U.S.C. § 8401 has expertise in viral pathogenesis and viral immunology, and has worked with models of alphavirus pathogenesis, including CHIKV and VEEV for (b)(6); (b)(3):7 U.S.C. § 8401 will be responsible for the overseeing the testing of candidate therapeutics for their ability to protect mice from acute CHIKV induced arthritis or VEE-induced viral encephalitis. (b)(6); (b)(3):7 will work in close collaboration with Drs. DeFilippis and Streblow, as well as the other research project leaders to set priorities for which drugs should be tested within the CHIKV or VEE models and to identify promising candidates that show promising in vivo potential so that those candidates can be taken forward for further optimization.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) Months. (b)(4) (b)(6); (b)(3):7 U.S.C. § 8401 has several years of experience working with pathogen infected mice under BSL-3 conditions. (b)(6); (b)(3):7 will work in close coordination with (b)(6); (b)(3):7 U.S.C. § 8401 to test candidate therapies for their ability to protection from CHIKV or VEE-induced disease. (b)(6); (b)(3):7 will administer therapeutics, perform CHIKV and VEE infections, and will monitor infected animals for disease signs and collect tissues to assess viral loads and virus-induced pathology. (b)(6); (b)(3):7 also directs the day to day operations of the BSL-3 facility where all work with CHIKV and VEE is conducted and will oversee the proper training and compliance of all individuals working within the BSL-3 facility.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) Months. (b)(4). (b)(6); (b)(3):7 U.S.C. has approximately (b)(6); (b)(3):7 U.S.C. § years of experience working with VEE and CHIKV infected mice under BSL-3 conditions. (b)(6); will be responsible for coordinating in vivo mouse studies and will be involved in the administration of candidate therapies and viral challenge studies. (b)(6); (b)(3):7 will also assist in collection of data to assess the impact of therapeutics on viral loads, virus-induced disease, and virus induced pathology within joint (CHIKV) and the central nervous system (VEE).

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) Months. (b)(4). (b)(6); (b)(3):7 U.S.C. § 8401 has experience working with alphaviruses and alphavirus molecular clones. (b)(6); (b)(3):7 will be responsible for testing candidate compounds for antiviral activity against CHIKV and VEE, determining what stage in the viral replication cycle the inhibitory compounds are acting, and determining whether resistance mutants arise against the compounds.

Fringe Benefits: Faculty/Staff: 22.883% Social Security and Retirement; $5,659FTE Health Insurance.

SUPPLIES

The evaluation of candidate therapies against either CHIKV or VEE requires the assessment of viral loads, evaluation of inflammatory cell infiltration and pathology within these tissues. Therefore funds are requested to cover the costs of tissue culture consumables (plastic ware, media, serum) required for the assessment of viral loads within the joints or CNS. We will also need to generate viral stocks, as well as generate infectious clones containing potential escape mutants and funds are requested to cover the costs of the molecular biology supplies needed for those purposes. We are also requesting funds to cover the cost of purchasing adult C57Bl/6 mice, which will be used for testing candidate therapies against both CHIKV and VEE, as well as funds to cover the cost of supplies needed to house these animals within our BSL-3 laboratory. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective gears, such as gloves, tyvek suits, and PAPRs.

TRAVEL

Funds are requested for the Project Leader, and 2 investigators to attend 1 scientific meeting to present findings and interact with other scientists in the field and to attend programmatic meetings.

OTHER EXPENSES

Equipment service contracts ($4,500): Several instruments in the (b)(6); (b)(3):7 Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology Costs ($5,000) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Federal F&A Cost Rate: In accordance to an agreement between DHHS and UNC dated May 16, 2012, the indirect cost rate is 52% of modified total direct costs.

A. COMPONENT COVER PAGE

Project Title: Project 3.3 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

MORRISON, THOMAS E

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, “hits” will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50 , CC50 , and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds.
Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 Project 3.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B.2 Project 3.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

1.Quinolones (SR-33394): Identify the mechanism of action for this class of compounds using in vitro assays. These will be tested for activity. Colorado will characterize VEEVTC83 virus that displays resistance to SR-34329 by utilizing sequence information generated by UNC to introduce the mutations into the infectious clone for VEEVTC83. SR plans to synthesize a new series based upon current SAR. Further SAR and testing will be dependent upon the results of the new analog series.
2.Tetralins-Benzo Annulenes (SR-33366): Additional SAR for this chemical series will be completed in order to optimize activity with stability and bioavailability. We plan to finish mechanism of action (MOA) studies for this compound series utilizing resistance phenotype information, in vitro assays and protein/compound binding. Since this compound series displays activity against a wide range of virus families; we will further characterize this property in order to determine the range of activity. In vivo pharmakokinetic (PK) analysis will be completed allowing us to determine dosing amount, route and timing for efficacy studies in our mouse models of CHIKV infection and disease.
3.VEEV 2015 HTS: SRI-36426 and SR-36427 were chosen for SAR due to their high activity and low toxicity profiles. SR will continue to provide the group with additional analog for these two scaffolds for SAR. PK analysis will be preformed on the most active compounds. Since SR-36426 is broadly active against 5 different Alphaviruses; we will determine the breadth of activity against other viruses. MOA studies should be completed for SRI-36426 and SR-36427.
4.CHIKV 2015 HTS: SR-36767 & SR36768 are the new leads for CHIKV but both compounds block a number of other Alphaviruses. We will continue to perform MOA and breadth of action studies for these two series. SR will continue to synthesize new analogs in order to optimize compound activity and stability. We hope to perform PK analysis and in vivo testing for these two series.
5.Project 1, 2, 4 Hits: We will continue to test additional compounds that are active against viruses from the other projects in order to identify broadly active compounds. We plan to determine the MOA for DENV compound SR-37014 because this was the most active compound identified in the cross-screens performed in 2016.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

Progress towards our goals is outlined for each Specific Aim:

SA1. HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screen: *VEEV HTS* identified 940 active samples and 8 out of 12 sent to OHSU had activity in NHDFs. *CHIKV HTS* identified 2,558 active compounds and 5 out of 11 were confirmed.
2. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified. OHSU tested 35 and found 4 actives against CHIKV. SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR and mode of action studies.
3. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication, Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). OHSU validated four anti-VEEV compounds as effective against CHIKV in these cells.
4. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate HTS and SAR including a new CHIKV strain expressing nano-Luciferase provided by UNC. Other recent isolates from Puerto Rico have been cloned and sequenced. These highly relevant strains may be used in subsequent validation experiments.
5. $VEEV_{TC83}$ has also been modified to encode nluc and is currently being validated at Colorado. $VEEV_{TC83}$-nLuc will be used by SR for SAR studies and the group for antiviral validation studies.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens and to identify the mode of action for the lead hits. To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has undertaken the optimization of specific assays that they will utilize to test lead compounds.
2. **Quinolones (SR-33394):** SR synthesized 89 analogs. OHSU tested them in virus reduction assays and found the active compounds SR-33394 (EC_{90}=0.77μM), SR-34329 (EC_{90}=0.12μM), SRI-36506 (EC_{90}=4.9μM) and SR-36959 (EC_{90}=0.78μM). All other analogs had decreased activity compared to SR-33394. Colorado generated a $VEEV_{TC83}$ virus that displays resistance to SR-34329, which is being sequenced by UNC.
3. **Tetralins-Benzo Annulenes (SR-33366):** SR synthesized >73 analogs of SR-33366 for SAR. SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC_{90}=0.45μM compared with SR-33366 (EC_{90}=3.2μM). Sequencing of UNC- and OHSU-derived resistance mutants identified changes in the macrodomain of NSP3. This finding is consistent with mode of action studies showing that SR-34963 blocks viral RNA synthesis at the level of subgenomic RNA synthesis. SR is performing crystallization/binding assays with NSP3. Analog SR-36429 (EC_{90}=1.5μM) showed better microsomal stability and PK activity and may be used for *in vivo* activity experiments. Additional recent analogs show activity and are under SAR. SR-34963 is also active against Flaviviruses (DENV, ZIKV), Coronaviruses, and Influenza virus.
4. **VEEV 2015 HTS:** OHSU confirmed 8 of 12 active hits including: SR-36415 (IC_{90}=0.77μM), SR-36416 (IC_{90}=0.35μM), SR-36420 (IC_{90}=0.13μM), SR-36421 (IC_{90}=0.11μM), SR-36423 (IC_{90}=0.22μM), SR-36424 (IC_{90}=0.06μM), SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM). SRI-36426 and 27 were chosen for further SAR. SR-36426 is active against 5 different Alphaviruses and blocks infection prior to viral RNA synthesis. SR-36427 is active against VEEV and Mayaro virus and blocks infection after RNA synthesis. Both work in $IRF3^{-/-}$ fibroblasts indicating that they do not function through IFN.
5. **CHIKV 2015 HTS:** OHSU confirmed 5 of 11 including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), SR-36767 (IC_{90}=0.09μM), and SR-36768 (IC_{90}=0.23μM). Two compounds (SRI-33001 and -36768) were active against 5 different Alphaviruses and SRI-36767 was active against 4 Alphaviruses. SR-36767 & -68 are the new leads for CHIKV and block infection prior to RNA synthesis. SR-33001 blocks viral replication at a step after viral RNA synthesis.
6. **Project 1, 2, 4 Hits:** DENV compound SR-37014 (IC_{90}=0.4μM) was active against CHIKV. SARS compounds SR-35742, -35894 and -36565 showed activity against VEEV but not CHIKV.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test *in vivo* efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease, including models of disseminated inflammation based on unique mouse strains from the Collaborative Cross Mouse Genetics Resource as well as the generation of a mouse-adapted CHIKV strain developed at Colorado that displays enhanced replication, dissemination, and pathogenicity in WT mice; 2) Intranasal inoculation of VEEV for neurological infections; 3) chronic CHIKV infection and joint disease in wild-type and immunodeficient mice; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of nonhuman primates. Each lab has unique models that they will use for testing lead compounds; this will increase our ability to quickly determine the *in vivo* efficacy profile for each lead.

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C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Thomas		Morrison		PD/PI	(b)(4); (b)(6)				27,684.00	7,752.00	35,436.00
Total Funds Requested for all Senior Key Persons in the attached file													
Additional Senior Key Persons:				File Name:								**Total Senior/Key Person**	**35,436.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			11,875.00	2,256.00	14,131.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	PRA				20,561.00	5,757.00	26,318.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**40,449.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**75,885.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: University of Colorado Denver

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	32,065.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Costs	40,000.00
Total Other Direct Costs	**72,065.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**147,950.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct Costs	55.5	147,950.00	82,112.00
		Total Indirect Costs	**82,112.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**230,062.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Project Budget Justification_Morrison.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel ($75,885)

Thomas E. Morrison, Ph.D., Co-Investigator (b)(4) **months).** Dr. Morrison has extensive experience and expertise in alphavirus pathogenesis, host immune responses to virus infection, and mouse models. Dr. Morrison will be responsible for the overall administration and direction of the project. He will be responsible for the overseeing studied designed to determine the mechanism of action of select compounds and for the testing of candidate therapeutics for their ability to protect mice from chronic CHIKV infection and disease. He will work in close collaboration with Drs. Heise, DeFilippis and Streblow, as well as the other research project leaders to set priorities for which drugs will be evaluated for mechanism of action and which will be tested within the CHIKV chronic disease model.

Katie Carpentier, Post-Doctoral (b)(4) **months).** Ms. Carpentier will perform mechanism of action studies in cells for select compounds. Ms. Carpentier also will perform CHIKV inoculations of mice, administer therapeutics, perform animal necropsies, and process tissues for quantification of CHIKV RNA and for the assessment of tissue pathology.

Nick May, Professional Research Assistant (b)(4) **months).** Mr. May will assist Mr. Hawman in testing therapeutics for their ability to protect mice from chronic CHIKV infection and disease. He will perform real time PCR analysis to evaluate viral RNA loads in virally infected tissues in the presence or absence of candidate therapeutics. In addition, Mr. May will perform mechanism of action studies in cells for select compounds.

Other significant contributors

Stephanie Montgomery, Ph.D., D.V.M., North Carolina State University, Raleigh, NC (no funds requested). Dr. Montgomery and Dr. Morrison have an active collaboration related to CHIKV-induced tissue pathology that was an important component of two previous publications. Dr. Montgomery, a veterinary pathologist with a doctorate in alphavirus biology, will provide expert analysis of histopathological changes in murine tissues.

Supplies ($32,065)

The evaluation of candidate therapies for the treatment of chronic CHIKV infection and disease in the mouse model requires stocks of infectious CHIKV. Therefore, we request funds for tissue culture consumables and transcription kits. To evaluate the effects of candidate therapies requires the isolation of RNA from specific tissues and the quantification of CHIKV RNA via qRT-PCR and preparation of tissues for evaluation of histopathologic changes. Therefore funds are requested to cover the costs of consumables such as Trizol, RNA isolation kits, reverse-transcriptase enzyme, and PCR reagents. We will also need to generate H & E stained tissue section for evaluation of histopathologic changes. In addition, we request funds for reagents and fees associated with high-throughput sequencing of any identified resistance mutant that emerge out of the animal experiments. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective equipment, such as gloves and tyvek suits.

Animals ($40,000)

The CHIKV chronic infection model utilizes three-to-four week old C57BL/6 mice. Therefore, we request funds to cover costs associated with the purchase of breeding pairs of mice, costs associated with maintaining an active breeding colony, and costs associated with housing experimental mice for up to 4-6 months under ABSL3 conditions.

A. COMPONENT COVER PAGE

Project Title: Project 4.2 Identification and characterization of novel drugs that target the influenza virus polymerase functions

Component Project Lead Information:

(b)(6); (b)(3):7 U.S.C. § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific aims: The overall goal of this project is to identify new therapies that target influenza virus replication. The global health burden of annual influenza epidemics coupled with the emergence of highly pathogenic strains of influenza virus has highlighted the urgent need for new effective treatments. A primary concern with the current drugs (amantadines and neuraminidase inhibitors) used to treat influenza is the development of resistance mutations that negate therapeutic benefit. Published evidence suggests that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy. The RdRp is responsible for a number of functions including 5'cap recognition, endonuclease activity, replication, transcription, and polyadenylation. Recently, cryo-EM reconstitution studies identified branched-ribonucleoproteins (RNPs) structures as putative replication intermediates and suggested a mechanism for viral replication by a second polymerase activity on the RNP template [1]. The second polymerase activity is believed to be a function of the polymerase complex. Clearly, the RdRp provides multiple functional domains that could be targets for antiviral drug therapy. Previous studies showed that mutations in the conserved regions of PB1 subunit of the polymerase complex produce inactive RNA polymerase [2]. We hypothesize that compounds that specifically target the polymerase complex might reduce the frequency of escape mutations, or promote escape mutants that are unfit for replication. We have recently identified potential hit compounds from previous HTS screens that significantly inhibit the influenza virus polymerase activity in an RdRp transient assay. These hit compounds were effective against three different strains of influenza viruses in CPE assays. Between Southern Research (SR) and the University of Alabama at Birmingham (UAB), all the necessary primary and secondary assays to perform HTS screening and identify compounds that specifically target the influenza virus polymerase activity have been developed. We propose the following specific aims:

Aim#1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.
Hypothesis and rationale: We hypothesize that by targeting the polymerase complex, we might reduce the frequency of mutational evasion because the mutants will be unfit for replication. Recent studies demonstrated that the nucleoside inhibitor T-705 induces lethal mutagenesis in H1N1 viruses in vitro resulting in a nonviable phenotype [3]. Targeting the influenza polymerase activity might prove more effective than targeting the viral glycoproteins because there are multiple proteins, as well as protein: protein and protein: RNA interactions, which could be targeted. Our goal is to identify compounds against the conserved regions of influenza virus polymerase subunits that might be effective against multiple viral strains.
Experimental strategy: The proposed transient influenza polymerase assay in aim#2 to identify anti-polymerase hits is not adaptable for HTS, and therefore a CPE-based assay will be used as a primary assay to screen novel libraries against influenza viruses. We will screen libraries that have not been previously screened for activity against the viruses covered in this proposal. These libraries are composed of highly diversified small molecules that contain novel and original drug-like features with distinct topologies and diverse functionalities.

Aim#2: Characterize the antiviral activity of hit compounds and identify anti-polymerase inhibitors.
Hypothesis and rationale: The existing hit compounds with polymerase inhibitory activity might target one or more subunits of the influenza virus polymerase. The CPE-based HTS screening will identify additional hit compounds that target all stages of the virus life cycle, including multiple functional domains of the influenza RNA polymerase. We have designed an experimental strategy that will focus our analysis on the hit compounds that block post-entry steps of viral infection.
Experimental strategy: We will use a variety of secondary assays to identify compounds that specifically inhibit the functions of the viral polymerase complex. Our proposed secondary assays will identify and exclude hit compounds that target viral entry and release, as well as interferon inducers. Following this exclusion process we will examine the remaining positive hit compounds in the transient polymerase assay. Once compound specificity for the viral polymerase is demonstrated, tertiary assays will be performed to determine the target within the polymerase complex.

Aim#3: Chemical optimization and determination of the in vivo efficacy of lead compounds.
Hypothesis and Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit the influenza replication complex. Chemical optimization of the effective scaffolds should generate compounds with greater efficacy, selectivity, and bioavailability.
Experimental strategy: The hit compounds from the HTS will be triaged and progressed as outlined in the Chemistry core. Compounds with the appropriate activity and pharmacokinetic properties will be evaluated using in-house mouse infection models.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Project 4.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: B4 Project 4 SR.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

As noted elsewhere, the HTS core completed the primary screen, and 892 hits were identified. The hits were evaluated at concentrations of 10 and 2 μM in a CPE-based assay in MDCK cells for activity against A/CA/10/2009 (H1N1) and A/Panama/2001/99 (H3N2), as well as for cytotoxicity at the same concentrations. The data are currently being analyzed. Confirmed hits from this analysis will be evaluated for potency and efficacy by determining the EC50 values in the MDCK-based CPE assay, and the extent of virus titer reduction (VTR) in the MDCK cells. Compounds active against both subtypes (EC50 ≤20 μM, CC50 ≥50, and VTR of ≥2-log10), will then be evaluated in the RNA-dependent RNA polymerase (RdRp) assay to determine if the activity is due to inhibition of the polymerase. Each compound will be tested at concentrations ranging from 0.39-50 μM, and the compounds with EC50 ≤20 μM in the RdRp assay will be selected for further analysis and preliminary structure-activity relationship studies. This will involve iterative cycles of compound design and synthesis of analogs, and their evaluation in MDCK-based CPE and VTR assays. The activity of promising compounds will then be tested in the primary human small airway epithelial cells using the NanoLuc influenza PATSN (H1N1), as well as against the avian (H5N1) subtype in MDCK cells. To identify the drug target for the active compounds, resistant mutants against each will be generated. The entire genomic RNA segments encoding the different polymerase subunits will be sequenced for each mutant to identify the precise drug target sites within the polymerase complex. In the unlikely event that no polymerase inhibitor could be identified, all active compounds (EC50 ≤20 μM, CC50 ≥50, and VTR of ≥2-log10 in the MDCK-based CPE and VTR assays) will be tested in Neuraminidase inhibition, Hemagglutination inhibition, and Virus Entry assays to identify their possible mode of action. Alternatively or additionally, resistant mutants will be generated and the entire genome will be sequenced for each mutant to identify the other potential viral targets.
With respect to future in vivo studies, The in vivo team purchased and documented accuracy of a microrectal thermometer for measuring body temperatures of mice as required by IACUC for influenza studies. Based on availability of nanoluc technology, we modified our regulatory files with IACUC and IBC to include imaging of infected mice through the UAB Imaging Core directed by Dr. Kurt Zinn. Once approved, mice will be purchased for lethality studies to determine the optimal dose for viral intranasal infections in BALB/c mice. Within weeks of the pilot study, we'll be prepared to initiate efficacy and toxicity studies of lead compounds.

B.2. Accomplishments under these goals:

B2.1. Evaluation of chemical analogs of the compound SRI 34518.

In the Year 2 report, we provided data indicating that SRI 34518, exhibited inhibitory activity against the pandemic (A/California/07/2009, H1N1), seasonal (A/Udorn/72, H3N2) and highly pathogenic avian (H5N1) subtypes of Influenza A virus (IAV) with EC_{50} values of 13.9, 5.03, and 7.01 µM, respectively. The compound was also found to be active in the minigenome-based IAV RdRp (polymerase) assay, whereby it inhibited the activity of IAV RdRp with an EC_{50} of ~3.0 µM. These data suggested that SRI 34518 is likely an IAV polymerase inhibitor with activity against multiple subtypes. However, structurally it does not provide a viable starting point. Therefore, 23 analogs of SRI 34518 were obtained and tested in the ELVIRA (reporter cell line) assay against the pandemic (H1N1) and seasonal (H3N2) IAV subtypes. Of these, only three compounds (SRI34993, SRI 34997, and SRI35058) were found to be active against both IAV subtypes with an EC_{50} <20 µM. Of these three, only SRI 34993 exhibited a lower EC_{50} and higher selectivity index (SI) than the parental compound SRI 34518. The compound SRI 34993 was then tested in the IAV minigenome RdRp assay and found to be inactive, indicating that SRI 34993 does not target the IAV polymerase; demonstrating that its anti-IAV activity is through a distinct mechanism. In addition, SRI 34993 was also tested against the avian (H5N1) IAV in then MDCK-based CPE assay and found to be inactive. Since the compounds tested here either had lower activity than the parental compound or did no inhibit the IAV polymerase, further work on this series is on hold at this time.

B2.2. Evaluation of compounds from Projects 1-3

One of the goals of the U19 is to identify potential targets or mechanisms with more broad applications. Too that end, a set of 18 compounds that exhibited activity against the viruses screened in Projects 1-3 were selected for screening as part of project 4. The compounds were tested against A/Udorn/72 (H3N2) subtype in the ELVIRA reporter cell line in a concentration response assay. Seven compounds (SRI 27298, 33361, 35894, 36418, 36422, 3678, 36772) inhibited IAV replication with an EC_{50} of <10 µM, and three compounds (SRI 35756, 36771, 36770) with an EC_{50} between 10 and 20 µM. Subsequently five compounds (SRI 33361, 36418, 36422, 36768, 36772) that had EC_{50} ≤5.0 µM in the ELVIRA assay were tested in the IAV RdRp assay to determine if they inhibited the IAV polymerase. Each compound was tested at concentrations ranging from 0.39 – 50.0 µM. All were active at (<3.0 - <6.0 µM) except SRI 36772. The compounds were also tested for their cytotoxicity 24 h and 72 h post-treatment in three different cell types including HEK293, A549, and MDCK. None of the compounds had any significant cytotoxicity 24 h post-treatment; however, SRI 33361 and SRI 36422 were cytotoxic in HEK293 cells with CC_{50} values of 2.66 and 2.21 µM, respectively, 72 h post-treatment. All 18 compounds were also evaluated in a CPE assay in MDCK cells against A/CA/10/2009 (H1N1), A/Panama/200I/99 (H3N2), and B/Florida/4/2006, using a concentration range of 0.016 – 50 µM. However, none of the compounds were found to active against Influenza A virus, and only three, SRI 35894, SRI 36768, and SRI 36770, displayed modest antiviral activity against the B strain of influenza virus with (EC_{50} values of 7.9 ± 12, 5.1 ± 5.9, and 10.2 ± 12.9 µM, respectively, and CC_{50} values that ranged between 21 and 24 µM). Therefore, due to inactivity against Influenza A virus and/or high cytotoxicity, no follow up work is planned for these compounds.

B2.3. Confirmation of antiviral activity of the 892 active compounds from the HTS screen.

The HTS core identified 892 compounds with confirmed antiviral activity in a dose response assay utilizing the ELVIRA reporter cell line. After Clustering and PANE filtration, these compounds were evaluated further in a 384-well CPE assay in MDCK cells at concentrations of 2 and 10 µM against A/CA/10/2009 and A/Panama/200I/99, and a cytotoxicity assay was completed with equivalent compound exposure. After data analyses, the top 14 hits that displayed >50% antiviral activity against both strains of Influenza A virus with <20% cytotoxicity at 10 µM have been selected for further analyses, as detailed in the plans for Year 4.

B2.4. Development of a 384-well influenza assay in primary small airway epithelial cells.

An assay was developed in 384-well plates using primary human small airway epithelial cells and the NanoLuc influenza strain A/California/04/2009 pdm (H1N1) PATSN. While this strain does not exhibit CPE in these cells, the NanoLuc activity expressed over two rounds of replication provided a reliable indicator of virus replication in these cells. This assay will prove to be valuable in downstream studies as it will allow further evaluation and confirmation of the lead compounds in primary human lung cells representing a more physiologically-relevant model system.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

Postdoctoral Fellows are active in the project. Southern Research has developed a comprehensive and strategic career Individual Development Plan (IDP) for Post-Doctoral Fellows within the Department of Infectious Diseases, Drug Discovery Division. Our training program is aimed at developing knowledge, skills, and reputation, together promoting the advancement of an independent career in drug discovery against infectious diseases. To ensure that trainees receive the knowledge and skills that are the necessary foundation of a scientific career, we offer training that gives them exposure to multiple key areas with which our faculty have established expertise including but not limited to: (a) biology and pathogenesis of infectious agents, (b) identification of novel drug targets, (c) development of pertinent assays for drug discovery, identification and development of molecular-targeted therapeutics, and (d) mechanism of action of drugs. Seminar series, journal clubs, manuscript and grant writing guidance ensure that each trainee will receive exposure to each of these areas (all of which are mainstays in the Department of Infectious Diseases); likewise, the qualifications of our faculty ensure that expertise will be adequate to offer guidance that is both current and accessible. Additionally, each trainee is strongly encouraged to submit at least one abstract annually for presentation at a national meeting which relates to his/her research areas. Progress of each trainee is evaluated on an annual basis. These evaluation meetings are used for defining key objectives and goals for progress for the upcoming year.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

Nothing to report

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	(b)(6); (b)(3):7 U.S.C. § 8401				Project Leader	(b)(4); (b)(6)				28,874.00	13,455.00	42,329.00
2.	Dr					Co-Project Leader					5,653.00	2,634.00	8,287.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **50,616.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
4	2 Biologist, 1 Life Sci. Researcher, 1 BSL3 Differential Pay (64 hours x $2)	(b)(4)			122,847.00	57,188.00	180,035.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**180,035.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**230,651.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ❍ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	9,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2017 **End Date*:** 02-28-2018

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	108,556.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. BSL3 Facility Fees	8,000.00
Total Other Direct Costs	**116,556.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**356,207.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries and Benefits	120.0	230,651.00	276,781.00
2. G & A - Total Direct Cost + OH	20.0	632,988.00	126,598.00
3. CFC - Salaries and Benefits	7.3	230,651.00	16,838.00
4. CFC - Total Direct Cost + OH	1.0	632,988.00	633.00
		Total Indirect Costs	**420,850.00**

Cognizant Federal Agency DHHS, Steven Zuraf, 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**777,057.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: BUDGET JUSTIFICATION- Proj 4.2 Year 4 120619.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION – Southern Research Institute

Section A – Personnel - Biochemistry and Molecular Biology

(b)(6); (b)(3):7 U.S.C. § 8401 Ph.D. (b)(4) months for Year 4), is a (b)(6); (b)(3):7 U.S.C. § 8401 in the Drug Discovery Division at Southern Research Institute (SR). He is also an (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 He is a virologist with nearly (b)(6); (b)(3):7 U.S.C. § 8401 of research experience. Over the years, (b)(6); (b)(3): has been involved in investigations related to various aspects of biology, pathogenesis, epidemiology, and evolution of various human viruses included in families *Bunyaviridae*, *Flaviviridae*, *Togaviridae* and *Filoviridae*. (b)(6); (b)(3): has extensive experience in BSL-3 and BSL-4 laboratories. In (b)(6); (b)(3):7 more recent investigations, (b)(6); (b)(3):7 deciphered the role of macropinocytosis in Ebola virus entry, and elucidated the important role of PI3K and CaMKII cell signaling pathways in the entry process. (b)(6); (b)(3): current research at SR is focused on discovery of small molecule antiviral compounds against Ebola, Zika, and encephalitic alphaviruses. (b)(6); (b)(3):7 has several peer-reviewed publications, and serves as Ad hoc reviewer for four journals in the field of virology. (b)(6); (b)(3):7 U.S.C. § 8401 will supervise and perform the experiments to determine the mode of action of hit compounds identified in primary and secondary screens. In particular, (b)(6) will supervise the work to exclude compounds that inhibit viral hemagglutinin and neuraminidase proteins, and identify the compounds that suppress viral replication by targeting the viral polymerase. This way the groups in this U19 program will focus their efforts at analyzing the post- entry inhibitors including the hit compounds targeting the viral polymerase. (b)(6); (b)(3):7 U.S.C. § 8401 will supervise experiments described in Aim#2 of this application. (b)(6); (b)(3):7 will assist in the design of *in vivo* experiments (Aim#3) and will assist in the determination of compound broad efficacy. (b)(6); (b)(3):7 will also coordinate the evaluation and presentation of data and manuscripts, and participate in Project meetings.

(b)(6); (b)(3):7 U.S.C. § 8401 laboratory (b)(4) months for Year 4) will be responsible for growing and determining the titer of influenza virus stocks to make them available in downstream confirmatory assays and mode of action studies (Aim#1 & 2). (b)(6); (b)(3):7 will also conduct secondary screening of hit compounds against different influenza virus subtypes. (b)(6); (b)(3):7 U.S.C. § 8401 will also perform antiviral biochemical assays related to Aim#1 & 2, and will assist with BSL-3 evaluations of antiviral compounds for broad efficacy (Aim#2). (b)(6); (b)(3):7 will also perform other biochemical assays including hemaglutination, and neuramindase assays.

(b)(6); (b)(3):7 U.S.C. § 8401 Ph.D., (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months for Year 4) will conduct secondary screening of hit compounds against an influenza virus *in vitro* polymerase assay (Aim#2d). (b)(6); (b)(3):7 will also perform the plaque reduction assays (Aim#2c) and the interferon assays (Aim#2b). Additionally, (b)(6); (b)(3):7 U.S.C. § 8401 perform experiments to determine the effect of hit compound on influenza virus protein expression (Aim#2f) and RNA expression (Aim#2e).

(b)(6); (b)(3):7 U.S.C. § 8401 Ph.D. (b)(4) months for Year 4), is a (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 has more than (b)(6); (b)(3):7 of experience in the field of bacteriology and phage biology at the (b)(6); (b)(3):7 U.S.C. § 8401 working in the fields of virology and bacteriology. (b)(6); (b)(3): was one of the developers of the live attenuated *Vibrio cholerae* vaccine 638, which is currently in clinical trials and has been shown to be well tolerated and immunogenic in humans. (b)(6); (b)(3):7 also discovered a new mechanism of

horizontal transmission of the cholera toxin genes mediated by the filamentous bacteriophage VGJφ in V. cholerae and has made other outstanding contributions to the field of filamentous phage biology. After (b)(6); (b)(3); joined Southern Research (b)(6); (b)(3); has been involved in several projects not only in the field of bacteriology, but also in virology, specifically in the study of the mechanism of entry of Ebola virus and the designing and development of a new assay for identifying inhibitors of the VEEV protease. In this last field (b)(6); (b)(3); is currently focused in the discovery of antiviral compounds against influenza less prone to develop viral resistance by targeting the RNA polymerase. (b)(6); (b)(3):7 U.S.C. § 8401 will supervise and perform secondary confirmatory assays on hit compounds found in the primary HT screening against IAV, specifically by implementing a qRT-PCR that has been optimized in his lab. (b)(6); (b)(3):7 will also supervise secondary assays for mechanistic characterization. In particular, cell entry assays to exclude hit compounds that act on viral entry. (b)(6); (b)(3):7 will assist in the designing of experiments to these aims and presentation of data and manuscripts, as well as participate in Project meetings.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months for Year 4) will continue developing and optimizing cell entry assays for influenza, west Nile, dengue, and SARS viruses (Aim#2a) and the interferon inhibition assay (Aim 2b). (b)(6); (b)(3):7 will also assist in screening the hit compounds in the entry assays, and in implementation of the qRT-PCR confirmatory assay for Influenza viruses.

Year 4 costs:

These include labor and materials for cell culture, biochemical assay performance, and general supply costs. BSL-3 laboratory charges are included for 18 days of operations and incubations. We request travel for 2 Project Leaders and 2 staff members to one scientific meeting per year to present our findings.

Supplies	$108,556
BSL-3 charges	$8,000
Travel	$9,000

Grant Number: 5U19AI109680-05
FAIN: U19AI109680

Principal Investigator(s):
Richard J. Whitley, MD

Project Title: Antiviral Drug Discovery and Development Center - Overall

Tabitha Payson
Univ of Alabama at Birmingham
1720 2nd Ave North
AB 1170-P
Birmingham, AL 352940111

Award e-mailed to: OSP-NGA@mail.ad.uab.edu

Period Of Performance:
Budget Period: 03/01/2018 – 02/28/2019
Project Period: 03/01/2014 – 02/28/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $7,112,904 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF ALABAMA AT BIRMINGHAM in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U19AI109680. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Vandhana Khurana
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5U19AI109680-05

Award Calculation (U.S. Dollars)

Salaries and Wages	$142,701
Fringe Benefits	$45,641
Personnel Costs (Subtotal)	$188,342
Consultant Services	$14,000
Materials & Supplies	$24,708
Travel	$37,659
Subawards/Consortium/Contractual Costs	$6,713,631
Publication Costs	$1,500
Equipment or Facility Rental/User Fees	$5,405
Federal Direct Costs	$6,985,245
Federal F&A Costs	$127,659
Approved Budget	$7,112,904
Total Amount of Federal Funds Obligated (Federal Share)	$7,112,904
TOTAL FEDERAL AWARD AMOUNT	$7,112,904
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$7,112,904

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$7,112,904	$7,112,904

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1636005396A6
Document Number:	UAI109680A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018
AI	8472315	$7,112,904

NIH Administrative Data:
PCC: M65B B / **OC**: 414P / **Released**: (b)(6) 02/28/2018
Award Processed: 03/01/2018 12:02:16 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5U19AI109680-05

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5U19AI109680-05

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in

the final progress report when applicable.

f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) U19AI109680. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last

recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience*.

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to: NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5U19AI109680-05

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **Oregon Health and Science University** in the amount of **$1,075,293** (**$636,239** direct costs + **$439,054** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Vanderbilt University Medical Center** in the amount of **$404,625** (**$256,092** direct costs + **$148,533** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Southern Research Institute** in the amount of **$4,067,680** (**$1,877,583** direct costs + **$2,190,097** F&A costs).

This Notice of Award (NoA) includes funds for activity with **Washington University** in the amount of **$245,327** (**$160,870** direct costs + **$84,457** F&A costs).

This Notice of Award (NoA) includes funds for activity with **University of North Carolina at Chapel Hill** in the amount of **$690,644** (**$454,371** direct costs + **$236,273** F&A costs).

This Notice of Award (NoA) includes funds for activity with **University of Colorado Denver** in the amount of **$230,062** (**$147,950** direct costs + **$82,112** F&A costs).

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No funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-045.html. Therefore, this award is issued at the committed level but reflects a rebudgeting of **$349** from the Personnel category to the **Other** category for **Project-5066**.

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This award is issued as a Cooperative Agreement, a financial assistance mechanism in which substantial NIH scientific and/or programmatic involvement is anticipated in the performance of the activity. This award is subject to the Terms and Conditions of Award as set forth in Section VI: Award Administrative Information of **RFA AI-12-044**, "**Centers of Excellence for Translational Research (CETR) (U19)**", posted date **11/23/2012**, which are hereby incorporated by reference as special terms and conditions of this award.

This RFA may be accessed at: http://grants.nih.gov/grants/guide/index.html

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This award may include collaborations with and/or between foreign organizations. Please be advised that short term travel visa expenses are an allowable expense on this grant, if justified as critical and necessary for the conduct of the project.

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Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that

changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Shan Liang
Email: shan.liang@nih.gov **Phone**: 301-761-7415 **Fax**: 301-493-0597

Program Official: Maureen J. Beanan
Email: beananm@mail.nih.gov **Phone**: 240-292-0999

SPREADSHEET SUMMARY
GRANT NUMBER: 5U19AI109680-05

Budget	Year 5
Salaries and Wages	$142,701
Fringe Benefits	$45,641
Personnel Costs (Subtotal)	$188,342
Consultant Services	$14,000
Materials & Supplies	$24,708
Travel	$37,659
Subawards/Consortium/Contractual Costs	$6,713,631
Publication Costs	$1,500
Equipment or Facility Rental/User Fees	$5,405
TOTAL FEDERAL DC	$6,985,245
TOTAL FEDERAL F&A	$127,659
TOTAL COST	$7,112,904

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	47%
F&A Cost Base 1	$271,614
F&A Costs 1	$127,659

A. OVERALL COVER PAGE

Project Title: Antiviral Drug Discovery and Development Center - Overall	
Grant Number: 5U19AI109680-05	**Project/Grant Period:** 03/01/2014 - 02/28/2019
Reporting Period: 03/01/2017 - 02/28/2018	**Requested Budget Period:** 03/01/2018 - 02/28/2019
Report Term Frequency: Annual	**Date Submitted:** 12/22/2017
Program Director/Principal Investigator Information: RICHARD J WHITLEY , MD AB **Phone number:** 205-934-5316 **Email:** rwhitley@peds.uab.edu	**Recipient Organization:** UNIVERSITY OF ALABAMA AT BIRMINGHAM UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave South, AB990 BIRMINGHAM, AL 352940001 **DUNS:** 063690705 **EIN:** 1636005396A6 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: MELINDA COTTEN 701 20th Street South Birmingham, AL 35294 **Phone number:** 2059758169 **Email:** mcotten@uab.edu	**Signing Official:** TABITHA PAYSON 1720 2nd Ave North AB 1170-P Birmingham, AL 35294 **Phone number:** 205-934-6798 **Email:** tpayson@uab.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** Yes If yes, previously reported: Yes

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The past 15 years have witnessed the emergence and re-emergence of several human viral infections of life threatening proportions, including diseases attributable to SARS coronavirus, highly pathogenic H5N1 influenza, pandemic 2009 influenza, monkeypox imported into the United States (US), West Nile virus (WNV) and dengue. Arguably, no efficacious therapy exists for most of these diseases and resistance is a threat to circulating influenza. Experimental approaches have been applied to each one of these diseases but with varying degrees of success.

The goal of this program is to form the Antiviral Drug Discovery and Development Center (AD3C) and identify compounds working though mechanisms that affect viral RNA replication and, importantly, to develop these leads in a translational manner to new human therapeutics. All four projects in this program are focused on viruses deemed critical to NIAID's focus on Emerging and Re-emerging Infectious Diseases related to biodefense. The projects perform High Throughput Screening utilizing unique compound libraries to identify novel chemical scaffolds with antiviral activity. Importantly, the projects report strong preliminary data that demonstrate the feasibility of performance of proposed mechanistic analysis of inhibitory compounds. In addition, all projects already have existing active compounds that will enter the drug discovery and development pathway at a later stage for evaluation.

The common theme of our application is targeting viral RNA replication. The experimental strategies designed by the four projects will provide a comprehensive analysis of the mechanism of action of the potential hit compounds. For example, it has been known for a long time that there are four consensus sequences that are conserved among the RNA-dependent RNA polymerases encoded by plus, minus and double stranded RNA viruses (1). The novel drug libraries with their diverse functionalities will allow the identification of compounds that might target conserved regions of the polymerase and thus yield broad-spectrum antiviral compounds. Based on the existing data in the literature and the preliminary data generated in the laboratories of the four groups we hypothesize that the development of drugs, which target enzymes such as polymerase and 2'O-methyl-transferase are rational approaches for the treatment of these viral diseases and will be more effective than targeting the surface glycoproteins. Resistance to drugs targeting the glycoproteins has frequently been reported. We hypothesize that viral escape mutants resulting from drugs targeting polymerase will be unfit for RNA replication, based on recent data in the literature. This data demonstrated that the mechanism of activity by the reported T-705 anti-polymerase drug is by inducing lethal mutagenesis in the polymerase protein, resulting in a nonviable virus unfit for replication. In AD3C, we will combine the virus-specific knowledge of leading virologists in the world with the high throughput screening and medicinal chemistry and lead optimization capabilities of Southern Research. The program's general specific aims are thus to:

1.Test viral targets essential to RNA replication in high-throughput-screening assays with unique chemical libraries to establish lead molecules for drug discovery.
2.Validate lead compounds in secondary and tertiary assays to confirm selectivity and mechanism of action as well as assure absence of off-target effects.
3.Probe the effects of lead molecules in representative animal models of targeted diseases and utilize such data to define impact on disease pathogenesis. Medicinal chemistry will optimize leads and further define platforms.

The individual projects all follow the general approach as described above, and are led by Drs. Jay Nelson (OHSU) and Michael Diamond (Washington University) to study compounds active against flaviviruses; Drs. (b)(6); (b)(3):7 (Vanderbilt) and Ralph Baric (UNC – Chapel Hill) to study compounds active against SARS-coronavirus; Drs. Dan Streblow (OHSU) and (b)(6); (b)(3):7 (UNC – Chapel Hill) to study compounds active against alphavirus and Drs. Ghalib Alkhatib, Jim Noah (Southern Research) and Rich Whitley (UAB) to study compounds active against influenza. All projects will extensively utilize the Screening Core and Medicinal Chemistry and Lead Development Core at Southern Research, which, with seven FDA approved drugs, has an outstanding track record of bringing drug discovery programs to clinical reality. All this will be coordinated out of the Administrative Core at UAB, which has extensive experience in drug discovery programs.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: AD3C EAB Report from Annual Meeting Oct 2017.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Umbrella B4 IDPs.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Year 5 will focus on the further development of chemical matter into lead compounds suitable for in vivo efficacy testing.

For some of these molecules, we have already initiated and will continue to identify how viruses will develop resistance to these new compounds, and map newly found resistance mutations to identify the compounds' molecular targets.

Select compounds will continue to be evaluated for microsomal stability and pharmacokinetic parameters such as plasma half-life, oral bioavailability and bio-distribution, to facilitate therapeutic studies in infection models in rodents.

Finally, progress in the last year should select compounds that would be suitable candidates to enter pre-IND enabling studies, going forward.

AD3C Annual Meeting -October 26- 27, 2017
External Advisory Board Report

Attendees: Dr. Kara Carter, Dr. George Painter, Dr. Pei-Yong Shi, EAB Board members, Dr. Maaike Everts, AD3C co-director, and Mary Wyatt Bowers, meeting recorder

The AD3C External Advisory Board met on October 27, 2017, following the project presentations to discuss and evaluate the progress of the four projects and three cores.

Overall comments:

Board members began with a generally positive evaluation of the overall program. However, although they have seen progress across all projects, particularly the corona virus/Project 2, they continue to see some of the same issues that were noted in last year's report. The projects and cores have mostly used cell based phenotypic screens with readouts such as CPE, an approach that limits the translational potential of the data. Going forward, for example for the grant renewal, the EAB would like to see more direct readouts that are focused on specific antiviral targets. They all agreed that in the coming year, the projects should focus on setting "go/no go" cutoffs, and further evaluate current leads with experiments such as surface plasmon resonance studies, soaking into or binding to crystals of the target virally encoded protein, or thermal shift studies. It was emphasized that physical parameters such as solubility of the compounds should be addressed much earlier in the discovery program(s) then they currently appear to be. It was suggested that investigators need to understand the pharmacodynamics/physiochemical profile of the compounds they want at the end and to "work backwards". There continues to be the need for more work on the biology versus chemistry; there should be understanding that secondary assays carried out in the project sites need to have SOPs, set standards, controls and confidence levels. Such assays should also include the lead molecule for the compound family in each run. Also, since this is translational research, investigators should consider the "pull from the market", e.g. go for what NIAID leadership and pharmaceutical companies can further develop, not necessarily follow what might be the most interesting basic science.

Evidence of synergy between the projects continues to be a very strong point. Compounds identified as active in one project were subsequently tested across projects, and there were hits identified that proved to be broad spectrum. The center has very accomplished investigators who get along well, and communicate frequently with each other and the Cores.

Project 1

Although progress has been made, members expressed concern that some unnecessary work is being done by the OHSU group on compounds without a clear way forward from a chemistry perspective. Investigators need to work with the chemistry core to decide what compounds to work on, and use well validated assays and approaches to, for example, determine mechanism of action. It is suggested to utilize expertise from the other projects to help with this. The concept of IFIT-related project is novel, but the approach has not been chemically and biologically validated for antiviral discovery. For the renewal, screening effort using the current approach should not be encouraged. Investigators should consider more innovative approaches that are different from the current one. For example, fast follow-up

approaches could be considered based on inhibitors in the literature; however, this approach requires strong chemistry support.

Project 2

This is clearly the most advanced of the projects and one that has made the best progress in the last year. Although the investigators did have the of advantage of starting with a strong lead from Gilead, they have taken advantage of the opportunity to develop the lead and get it to a potentially marketable product. Investigators should continue to advance their work with epithelial cells and mouse models. The collaboration with Gilead should be used as a model for bringing more mature compounds into the program, particularly when looking at projects for the upcoming grant competition. Also, since the compound is also active in Ebola, it can be considered to be a broad spectrum antiviral agent.

Project 3

This project has made good progress over the past year although it appears to have either slowed down somewhat since last year, or the other projects have caught up. The EAB thinks it was a waste of time and resources to test a compound with extremely poor solubility in an in vivo model of Chikungunya infection. The results were unclear as the compound was either inactive or failed to reach critical concentrations at the site of action. The investigative team is excellent though. Since NIAID director, Dr. Fauci, mentioned Alphaviruses in a recent institute review, it is clearly an important project to have in the program.

Project 4

Due to several factors, this project has basically gone through a “restart” this past year and the UAB PI, Dr. Mark Prichard, has now taken on a more active role. Board members noted that he has a good relationship with NIAID and has been making good progress. This is the one project that may want to do some additional screening in the immediate future, but it should be very targeted and directly focused on the polymerase, which is what NIAID wants. Investigators should also have a clear concept of what they are looking for and what they are trying to do – a concept of the target molecule’s attributes. For example, what are the liabilities of the Vertex compound? Identify those and then structure your program to overcome them. Also, the investigators should always make sure to use appropriate positive and negative control compounds in all the assays (this would exclude the use of T705, for example).

Core A - Admininstrative

Promoting communication among the projects continues to be a strong point. During the coming year, leadership should focus on making sure that contractual and IP issues are clear so that next stages of development can take place, particularly for Project 2 where existing Gilead compounds are being worked on and more advanced leads can be obtained from other institutions, such as the Emory Institute for Drug Discovery. Since this will be the final year of the grant, the Core should also review the original RFA to define the final deliverables and output, and begin consideration of projects for the recompetition.

Core B - HTS

While the work done in previous years was good and needed, board members clearly stated that the time is past for doing the major, large scale screens for any of the projects. Fragment based and/or SPR screening may be useful, but any compound libraries should be narrowed down and any additional screening should be very focused with a defined mechanism/target.

Core C – Med Chem

The lead chemist at Southern Research has a good relationship with Project investigators, particularly the Project 3 PI, and did provide the type of data that is needed for some of the projects. However, board members expressed concern that the presentations by SR chemists showed no innovative chemical platforms and appeared to be somewhat rudimentary compared to that being done by industry. It is disappointing that several of the lead series could not overcome the problem with solubility and/or metabolic stability.

Supplemental project

Since the supplemental funds provided were limited to screening for Zika only with no additional activities, it was not possible to further evaluate this project. Board members did note that hits found during the Zika screen were also tested against Dengue, and represented a good effort to find a broad spectrum anti-Flavivirus compound.

IDPs:

Please refer to the Project descriptions for Individual Development Plans used by the respective institutions with which the trainees are affiliated.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Sali TM, Pryke KM, Abraham J, Liu A, Archer I, Broeckel R, Staverosky JA, Smith JL, Al-Shammari A, Amsler L, Sheridan K, Nilsen A, Streblow DN, DeFilippis VR. Characterization of a Novel Human-Specific STING Agonist that Elicits Antiviral Activity Against Emerging Alphaviruses. PLoS pathogens. 2015 December;11(12):e1005324. PubMed PMID: 26646986; PubMed Central PMCID: PMC4672893.
Complete	McCarthy MK, Morrison TE. Persistent RNA virus infections: do PAMPS drive chronic disease?. Current opinion in virology. 2017 April;23:8-15. PubMed PMID: 28214732; PubMed Central PMCID: PMC5474173.
Complete	(b)(6); (b)(3):7 U.S.C. § 8401

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Component(s)	Country	SS
(b)(6)	Y	WHITLEY, RICHARD J.	AB,MD	PD/PI	(b)(4); (b)(6)				Admin Core-5064 (Administrative Core - Core A)		NA
	N	Eagar, Jessica	MS	Technician					Project-5068 (Project 4.1 Identification… ase functions)		NA
	Y	Everts, Maaike	PHD	Co-Investigator					Admin Core-5064 (Administrative Core - Core A)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	PHD	Co-Investigator					Project-5072 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	PhD	Staff scientist (Doctoral level)					Project-5073 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	Botto, Sara	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position					Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	Y	DeFilippis, Victor Robert	PHD,MS	Co-Investigator					Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Quenelle, Debra	DVM,PhD	Co-Investigator					Project-5068 (Project 4.1 Identification… ase functions)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position					Project-5075 (Project 4.2 Identification… ase functions)		NA
	Y	Hirsch, Alec	BA,PHD	Co-Investigator					Project-5065 (Project 1.1 Identification… ug Candidates)		NA
	N	McGraw, Jennifer	BS	Graduate Student (research assistant)					Project-5073 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	PHD	Co-					Project-5065		NA

(b)(6); (b)(3):7 U.S.C. § 8401		(b)(6); (b)(3):7 U.S.C. § 8401		Investigator	(b)(4); (b)(6)		(Project 1.1 Identification… ug Candidates)		
	N	(b)(6); (b)(3):7 U.S.C. § 8401	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-5072 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Carpentier, Kathryn Semmens	BA,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-5074 (Project 3.3 Novel Therapeu… Alphaviruses)		NA
	Y	Prichard, Mark Neal	PHD,BS, MS	Co-Investigator			Project-5068 (Project 4.1 Identification… ase functions)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	Ph.D.	Co-Investigator			Project-5072 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-5072 (Project 2.2 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401	PhD	Staff scientist (Doctoral level)			Project-5073 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	Zhang, Sixue	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Karyakarte, Shuklendu	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Nguyen, Theresa	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	Y	Sheahan, Timothy P.	PhD	Co-Investigator			Project-5072 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Zhang, Rong	PHD,BS	Postdoctoral Scholar, Fellow, or			Project-5071 (Project 1.2 Identification…		NA

				Other Postdoctoral Position	(b)(4); (b)(6)		ug Candidates)		
	N	Ahmed, Kaleem S	PhD	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Ando, Takeshi	MD/PhD	Adj. Research Associate Professor			Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Bao, Donghui	PhD	Research Scientist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Bonin, Kiley		Non OHSU Student Worker			Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Bowers, Mary Wyatt	MA	Admin Core Business Manager			Admin Core-5064 (Administrative Core - Core A)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	B.S.	Supervisor Compound Management			Core-5069 (Screening Core - Core B)		NA
	N	Davis, Sara	B.S.	Admin Core Administrative Coord.			Admin Core-5064 (Administrative Core - Core A)		NA
	N	Deimler, Robert	PhD	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Denton, Michael	BS	Sr. Research Assistant			Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	MS	Project Manager			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Garzan, Atefeh	PhD	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Hancock, Meaghan H	BS	Research Asst. Professor			Project-5065 (Project 1.1 Identification… ug Candidates)		NA

	N	(b)(6); (b)(3):7 U.S.C. § 8401		Biologist	(b)(4); (b)(6)		Core-5069 (Screening Core - Core B)		NA
	N	Keith, Kathy	MS	Research Lab Supervisor			Project-5068 (Project 4.1 Identification… ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		Senior Research Specialist			Project-5066 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	Madadi, Nikhil	PhD	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Manuvakhova, Anna	B.S.	Advanced Bio IT Specialist			Core-5069 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	MS	Biologist			Project-5075 (Project 4.2 Identification… ase functions)		NA
	N	May, Nick	B.S.	Professional Research Assistant			Project-5074 (Project 3.3 Novel Therapeu… Alphaviruses)		NA
	N	Moukha-Chafiq, Omar	PhD	Research Scientist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Parkins, Christopher	M.S.	Research Associate			Project-5065 (Project 1.1 Identification… ug Candidates)		NA
	N	Poon, David	BS	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	BS	Biologist			Project-5075 (Project 4.2 Identification… ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	M.S.	Advanced Bio IT Specialist			Core-5069 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	M.S.	Supervisor HTS Center			Core-5069 (Screening Core - Core B)		NA
	N	Rodzinak, Kevin	BS	Research Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA

	N	(b)(6); (b)(3):7 U.S.C. § 8401	M.S.	Biologist	(b)(4); (b)(6)		Core-5069 (Screening Core - Core B)		NA
	N	Smith, Patricia		Senior Research Associate			Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Stevens, Laura J		Senior Research Specialist			Project-5066 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	Struthers, Hillary	BA	Research Assistant I			Project-5065 (Project 1.1 Identification… ug Candidates)		NA
	N	Tanner, Samuel	MS	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Torres, Regan		Research Assistant			Project-5065 (Project 1.1 Identification… ug Candidates)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	M.S.	Research Scientist			Core-5069 (Screening Core - Core B)		NA
	N	Venukadasula, Phanindra	PhD	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Wei, Han-Xun	PhD	Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	West, Ande	BS	Research Specialist			Project-5072 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		Research Assistant			Project-5066 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	B.S.	Biologist			Core-5069 (Screening Core - Core B)		NA
	N	Wu, Mousheng	PhD	Research Chemist			Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Zhang, Wei	PhD	Research Scientist			Core-5070 (Medicinal Chemistry and		NA

									Le…Core - Core C)		
(b)(6)	Y	Basu, Arnab	Ph.D.	Project Leader	(b)(4); (b)(6)				Project-5075 (Project 4.2 Identification… ase functions)		NA
	Y	Augelli-Szafran, Corinne	PhD	Co-Investigator					Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401	PhD	Research Associate Professor					Project-5066 (Project 2.1 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	MD	Project 2.1 Project Leader					Project-5066 (Project 2.1 Inhibitors of … Therapeutics)		NA
	Y		BA,PHD	Project 3.2 Consortium P.I.					Project-5073 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	White, James	PhD	Research Instructor					Project-5071 (Project 1.2 Identification… ug Candidates)		NA
	Y	(b)(6); (b)(3):7 U.S.C. § 8401	PHD,BS	Screening Core B Project Leader					Core-5069 (Screening Core - Core B)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401		Co-Project Leader					Project-5075 (Project 4.2 Identification… ase functions)		NA
	Y	Diamond, Michael S	PHD,MD, BA,MD,P HD	Project 1.2 Project Leader					Project-5071 (Project 1.2 Identification… ug Candidates)		NA
	Y	Suto, Mark J	BS,PHD	Co-Investigator					Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	Y	Nelson, Jay A	BS,PHD,B S,BOTH	Project 1.1 Leader					Project-5065 (Project 1.1 Identification… ug Candidates)		NA
	Y	Pathak, Ashish Kumar	PHD,MS, BS	Project Leader					Core-5070 (Medicinal Chemistry and Le…Core - Core C)		NA
	Y	Baric, Ralph S	PHD,BS	Project 2.2 Project Leader					Project-5072 (Project 2.2 Inhibitors of …		NA

									Therapeutics)		
(b)(6)	Y	Whitley, Richard J.	AB,MD	PD/PI	(b)(4); (b)(6)				Project-5068 (Project 4.1 Identification… ase functions)		NA
	Y	Streblow, Daniel N	PHD,BS	Project 3.1 Project Leader					Project-5067 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	Y	Morrison, Thomas E	MA,PHD, BA,BA	Project 3.3 Leader					Project-5074 (Project 3.3 Novel Therapeu… Alphaviruses)		NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: AD3C Other Support Combined.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OMB No. 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED

PHS 398 OTHER SUPPORT

WHITLEY, R.J.

ACTIVE

HHSN272201100034C (Whitley, Kimberlin Co PI) 9/28/11-8/15/20
HHS-NIH-NIAID $3,385,690
(b)(4)
An adaptive sequential study evaluating prevention of neonatal HSV: Detection of maternal shedding at delivery followed by preemptive antiviral therapy in exposed neonates.
In this project, a multi-institutional team of investigators, known as the Collaborative Antiviral Study Group (CASG), will validate the GeneXpert® HSV polymerase chain reaction (PCR) system by comparing it against standard quantitative PCR and routine viral culture.

HHSN272201100035C (Whitley, Kimberlin Co PI) 9/28/11-8/15/20
HHS-NIH-NIAID $2,065,894
(b)(4)
A Phase II 6 Weeks oral Valganciclovir vs Placebo in infants with Congenital CMV infection and hearing loss.
In this project, the CASG will identify infants and toddlers with SNHL and then will test these patients' DBS obtained during the neonatal period for CMV DNA by PCR.

HHSN272201100036C (Whitley, Gnann Co PI) 9/28/11-8/15/20
HHS-NIH-NIAID $2,188,470
(b)(4)
Natural History of Infection Caused by BK Virus (and other Opportunistic Viral Pathogens) in Renal and Renal-Pancreas Transplant Recipients
The primary objective of the study is to determine the natural history of kidney transplant patients with BKV viremia.

HHSN272201100037C (Whitley, Kimberlin Co PI) 9/28/11-8/15/20
HHS-NIH-NIAID $1,779,753
(b)(4)
A PK/PD and Resistance Evaluation of Intravenous Ganciclovir in Premature Infants
In this project, a multi-institutional group of investigators, known as the Collaborative Antiviral Study Group (CASG), will enroll premature subjects who are being treated clinically with intravenous ganciclovir for postnatally or congenitally acquired CMV disease.

HHSN272201100038C (Whitley, Kimberlin Co PI) 9/28/11-8/15/20
HHS-NIH-NIAID $1,967,277
(b)(4)
Adaptive study of CMX-001 in infants with Neonatal Herpes Simplex Virus (HSV)
In this project, a multi-institutional team of investigators, known as the Collaborative Antiviral Study Group (CASG), will define the pharmacokinetics (PK) and concentration response relationship of CMX001 in neonates with HSV CNS disease.

1U19AI109680-03 (Whitley, PI) 3/1/14-2/28/19
HHS-NIH- NIAID $1,210,504 Admin core; $780,179-Proj 4
(b)(4)
Center for Antiviral Drug Discovery and Development-UAB
Role: Administrative Core: PI, and Project Director; Project 4.0 – Influenza: Co-PI
Consortium which focuses on development of antiviral therapeutics for four major categories of emerging infectious diseases including Flaviviruses, Corona viruses, Alphaviruses and Influenza.

(b)(4)

U54TR001368-01 (Kimberly) 9/01/15 – 8/31/20
NIH/NCATS $6,324,075 (UL1, KL2, TL1)
UAB Center for Clinical and Translational Science (CCTS)
The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS supports this overall mission in a highly integrative network of relationships. Five strategic priorities are: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability.
Role: Co-Investigator, Project Leader

(b)(4)

NCI 5P30CA013148 (Partridge, PI) 4/01/16 – 03/31/21
NIH-NCI $420,000 (Administrative Core)
Comprehensive Cancer Center Core Support Grant
The major goal of this project is to support cancer research with emphasis on interdisciplinary efforts for the Comprehensive Cancer Center.
Role: Co-Investigator

(b)(4)

UM1 AR065705 (Curtis, Winthrop, MPI) 9/01/14 – 8/31/19
NIH/NIAMS $437,212 Annual Direct Costs
Safety and Effectiveness of Live Zoster Vaccine in Anti- TNF Users (VERVE)
The Varicella zostER VaccinE (VERVE) trial is a randomized, double-blind, placebo-controlled large pragmatic trial to evaluate the immunogenicity, safety, and longer-term effectiveness of the live HZ vaccine in arthritis patients receiving anti-TNF therapy
Role: Advisor

(b)(4)

HHSN272201600017C (Kimberlin, PI) 7/1/16-6/30/21
NIH/NIAID $9,975,877
A Phase II, Single-Stage, Single Arm Investigation of Oral Valganciclovir Therapy in Infants with Asymptomatic Congenital Cytomegalovirus Infection.
Role: Co-Investigator

COMPLETED

(b)(4)

N01-AI-30025 (Whitley, PI) 8/1/03-12/01/13
NIH-NIAID $3,719,919
Clinical Trials for Antiviral Therapies
This contract serves to facilitate the development of promising therapies for treating severe, acute, and chronic human viral diseases that are deemed medically and scientifically important by the NIH-NIAID. This program will facilitate advances in clinical antiviral therapy by rigorously evaluating the efficacy and safety of new therapeutic regimens for serious viral diseases in adult and pediatric patient populations.

(b)(4)

3 U54 A1057157 (Sparling-UNC PI) 3/01/09–2/28/14
NIH $31,000
SERCEB Southeast Regional Centers for Excellence for Biodefense
This grant is jointly submitted by investigators in the Southeastern United States and researches new ways to contribute to the national effort in biodefense, as well as to study emerging infectious diseases that threaten both our country and our world.
Role: Co-Investigator.

2K12HD043397-09 (Stagno, PI) 3/10/08-11/30/13 (b)(4)
NIH $349,821
Pediatric Physician Scientist in Translational Molecular Biology
The goal is to enhance the mentored research experience with a foundation of research techniques and approaches. Role: Co-Investigator.

1U54 RR024376-05 (Kimberly, PI) 7/1/08-4/30/14 (b)(4)
NIH $232,220
UAB Center for Clinical and Translational Science (CCTS)
The UAB Center for Clinical and Translational Science (CCTS) will transform the UAB environment by building productive and efficient interdisciplinary research teams through educational ingenuity, regulatory reorganization, resource coordination, and methodological innovation. Its mission is to develop a transformative infrastructure that spans the spectrum from pre-clinical research to bench-to-bedside translation (T1 research) to community implementation (T2 research).
Role: Co-Investigator, Project Leader

1UL1RR025777-01(Kimberly, PI) 05/01/14-4/30/15 (b)(4)
NIH $80,109
UAB Center for Clinical and Translational Science (CCTS) supplement
The UAB Center for Clinical and Translational Science (CCTS) Drug Discovery project continuation which seeks to provide program for academic drug development in fulfillment of the overall mission to develop a transformative infrastructure that spans the spectrum from pre-clinical research to bench-to-bedside translation (T1 research) to community implementation (T2 research).
Role: Co-Investigator, Project Leader

5P01-CA 071933-15 (Whitley, PI) 7/1/09-6/30/15 (b)(4)
NIH-NCI $218,869
Engineered HSV for the Treatment of Malignant Glioma
The long-term objective of this program project grant, in collaboration with Dr. Bernard Roizman at the University of Chicago, was the design and testing of novel recombinant herpes simplex viruses as vectors of noxious genes for the selective destruction of human glioma cells.

OTHER SUPPORT
CORINNE E. AUGELLI-SZAFRAN

ACTIVE –

1R21NS096531-0, Hjelmeland (PI) 06/15/2016 – 03/31/2018 (b)(4)
NIH/NINDS $65,000
Identification of Glucose Transporter 3 Inhibitors for Glioblastoma Treatment
Goal: This is a collaborative drug discovery project which utilizes molecular modeling, ligand-based and structure-based virtual screening, computer-aided drug design, chemical synthesis and biological evaluations, to identify small molecule GLUT3-selective inhibitors for the treatment of GBM.
Role: Co-PI

(b)(4) Roberson (PI) 6/01/2015 – 06/30/2018 (b)(4)
$26,034
A New Approach to Targeting Tau in Alzheimer's disease by Inhibiting Its Interaction With Fyn
Goal: Development of Inhibitors of the Tau-Fyn Interaction for the Treatment of Alzheimer's disease. This project aims to develop inhibitors of the Tau–Fyn interaction for treatment of Alzheimer's disease and represents a new therapeutic strategy for the disease.
Role: Co- Investigator

HHSN272201400010I, Ptak (PM) 08/15/2017 – 8/14/2018 (b)(4)
NIAID Task Order 28 $275,834

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator.

HHSN272201400010I, Ptak (PM) 09/15/2016 – 9/14/2018 (b)(4)
NIAID Task Order 24 $ 326,951
In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
Goal: NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator

1U19AI109680, Whitley (PI) 03/01/2015 – 02/28/2019 (b)(4)
NIAID $1,046,665
UAB 000502793-011
Antiviral Drug Discovery and Development Center.
Goal: Development of antiviral drugs for the treatment of emerging and reemerging infections. Specifically, the focus will be on flaviviruses, alphaviruses, corona viruses and influenza. The goal is to identify compounds working through mechanisms that affect viral replication and develop these leads in a translational manner to new human therapeutics. As described above, each of the projects is focused on a viral family deemed critical to NIAID's focus on Emerging and Re-emerging Infectious Diseases related to biodefense. Role: Co-Investigator

Suto (PI) 9/14/2015 – 9/13/2020 (b)(4)

(b)(4)

$1,634,391

Identification of Therapeutics for CF
Goal: The goal is to identify novel read-through drugs for the treatment of cystic fibrosis. Initially, a large high-throughput screen will be run and the compounds further evaluated in several mechanistic assays. Lead optimization and profiling of the compounds will be initiated to identify a preclinical candidate.
Role: Co-Investigator

(b)(4)

1R01AI121364-01A1, Wolschendorf (PI) 7/1/2016 – 6/30/2018
NIAID/NIHDHHS $218,053
UAB 000511529-002
HTS for coper-activated inhibitors against MRSA.
Goal: Conduct a novel HTS strategy to reveal and advance new scaffolds with copper-related antibacterial activities for therapeutic applications. Harnessing the antibacterial properties of copper ions for therapeutic applications can best be achieved using small molecules with affinities for metallic ions, affording the resulting metal complex antibacterial properties.
Role: Co-Investigator

PENDING

(b)(4)

(b)(4)

OVERLAP - None

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 (b)(4) mon
NIH/NIAID $1,572,931

Characterization of novel genes encoded by RNA and DNA viruses

Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

Grants Management Specialist: Regina E. Kitsoulis, NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: kitsoulisre@niaid.nih.gov

Specific Aims: Determine the role of uncharacterized genes in highly pathogenic human infection (Project 1) Aim 1. Biochemical characterization of novel coronavirus genes, including hypothetical genes/noncoding RNA/unknown ORF. Aim 2. Defining novel functions in virus replication in vitro. Aim 3. Defining novel functions in virus pathogenesis in vivo. All 3 classes of genes will be characterized for their ability to modulate interferon signaling (Project 1), apoptosis (Project 2), inflammasomes (Project 3), and p53 signaling (Project 3).

U19-AI100625 (MPI: Baric/Heise) 08/05/12-08/31/22 (b)(4) mon
NIH/NIAID $2,437,629

Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross

Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

Grants Management Specialist: Gregory P. Smith, NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: gsmith@niaid.nih.gov Phone: 240-669-2993

Specific Aims Project 1 (CoV): Aim 1: Identify polymorphic genes regulating variation in Virus-induced innate and adaptive immunity.; Aim 2: How do specific polymorphic genes or combinations of genes impact Virus-induced immunity and pathogenesis?; Aim 3: Do polymorphic genes affecting Virus-induced immunity across viruses or impact other immune responses in mice and humans?

P01AI106695 (PI: E. Harris) 07/29/15-06/30/18 (b)(4) mon
UCB/NIH $279,165

Protective immunity following dengue virus natural infections and vaccination

We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).

Role: Co-Investigator

Grants Management Specialist: Mark Challberg NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: mchallberg@niaid.nih.gov

Specific Aims: 1) Improve understanding of what constitutes protective adaptive immunity in 1° and 2° DENV infections and in naïve and previously DENV-exposed recipients of a dengue TVLAV, which can inform future vaccine formulations; 2) Identification of natural and vaccine-induced B cell/antibody and CD4+/CD8+ T cell correlates of protection that can be used to assess existing and future vaccines; 3) Identification of potential therapeutic monoclonal antibodies and T cell peptide vaccines; and 4) Mapping of novel epitopes and generation of recombinant viruses that can serve as new epitope-specific diagnostic tools.

R01 AI 107731 (PI: de Silva) 08/05/13-07/31/18 (b)(4) mon
NIH/NIAID $621,124

Molecular Basis of Dengue Virus Neutralization by Human Antibodies

These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator
Grants Management Specialist: Mark Challberg NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: mchallberg@niaid.nih.gov
Specific Aims: The humoral immune response to a pathogen is derived from long-lived plasma cells (LLPCs) that contribute to circulating Abs and a memory B cell (MBC) pool that is activated upon re-exposure to the pathogen. While it is known that DENV infection stimulates robust LLPC and MBC responses, specific properties of Abs derived from these two compartments have not been characterized in detail. Based on recent findings from our group and others, we propose to test the hypothesis that new epitopes created by close packing of envelope (E) protein molecules on the viral surface are the main target of the human DENV serotype-specific neutralizing Ab response. People exposed to primary DENV infections develop long-term serotype specific neutralizing Ab responses whereas secondary infections result in serotype cross neutralizing responses. We propose that second infections preferentially activate somatically mutated MBCs expressing Abs that bind with high affinity to 2 or more serotypes and are capable of cross neutralizing serotypes. We propose three specific aims to characterize neutralizing antibodies derived from MBC and LLPCs after primary infections (Aims 1 and 2) and secondary infections (Aim 3).

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/18 (b)(4) mon
Univ of Wisconsin/NIH $87,000
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking "snapshots" of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Investigator
Grants Management Specialist: Marciele M. Degrace NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: degracemm@mail.nih.gov
Specific Aims: Aim 1. We will perform MERS-CoV infection of human Calu3 airway epithelial cells to study virus-host interaction networks. Aim 2. We will perform MERS-CoV infection of primary human lung cells to define unique and shared virus host interaction networks.

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4) mon
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator
Grants Management Specialist: Maureen Beanan NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: beananm@mail.nih.gov
Specific Aims Project 2: 1. To identify and develop inhibitors of CoV high-fidelity replication. 2. To identify and develop inhibitors of CoV RNA capping activity. 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors.

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4) mon
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

Grants Management Specialist: Tina Parker NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: parkerti@mail.nih.gov

Specific Aims Project 2: In Aim 1, we will develop platforms to recover novel respiratory viruses and identify diagnostic and prognostic indicators of severe lung disease by infecting primary human lung cells. In aim 2, we use the Collaborative Cross Mice to develop new animal models of human disease and to define conserved genomic signatures that correlate with etiology and disease severity and then validate the role of these biomarkers in models of outbred human populations infected with different high and low path respiratory viruses. In Aim 3, the goal is to use functional genomics and computational biology to not only diagnose virus etiology and forecast disease severity in the lung, but in parallel, develop a highly portable screening platform that rapidly identifies and then validates the lead compounds that attenuate disease severity in robust models of outbred human populations.

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 (b)(4) mon
NIH/NIAID $3,683,050

Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis

The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Grants Management Specialist: Carine Normil NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: carine.normil@nih.gov

Specific Aims: In Aim 1, we study the biochemistry and structure of MERS-CoV and HKU4 bound to various species receptor-binding domains (RBD). In Aim 2, we study Spike processing by bat and human entry proteases. In Aim 3, we study HKU4 and MERS-CoV pathogenesis using transgenic mice and experimental evolution. The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

(b)(4) (PI: Baric) 01/08/16-01/07/19 (b)(4) mon
$1,243,048

In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines

To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

Contracting Officer: (b)(4)

Specific Aims: This proposal formulates a collaboration between Dr. Ralph Baric and Dr Aravinda de Silva at the University of North Carolina and (b)(4), which was prompted by recent technological advances that their groups have made; i) in studying the specificity of human B-cell responses to quaternary structure epitopes in naturally infected (with DENV) individuals, and ii) developing virus constructs where the quaternary epitopes can be transplanted between DENV serotypes.

R01-AI125198 (PI: de Silva) 05/04/16-04/30/21 (b)(4) mon
NIH/NIAID $1,153,997

Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy

Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines.

Role: Co-Investigator

Grants Management Specialist: Mark Challberg NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: mchallberg@niaid.nih.gov

Specific Aims: The central hypothesis of this proposal is that the quality (Ab epitope specificity) rather than total quantity of cell-culture neutralizing Abs is a better predictor of DENV vaccine performance in human populations. Moreover, as the DENV complex has 4 serotypes and vaccines will be used in populations with a mix of naïve

and partially immune individuals, immune assays based on a single epitope are unlikely to predict efficacy against the 4 serotypes. This project is grounded on studies in our laboratories to understand protective and pathogenic Ab responses in people exposed to natural DENV infections. We have discovered new quaternary structure Ab epitopes linked to protection and developed reagents (human monoclonal Abs, recombinant DENVs) and assays that precisely measure Ab epitope-specific responses in human sera.

60045042 (PI: Saif/Wang) 02/01/15-01/31/18 (b)(4) mon
Ohio State Univ/USDA $44,804
Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs
Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.
Role: Subcontract PI
Contracting Officer: Margo Holland Awards Management Division, USDA/NIFA, Washington, DC 20250-2271
Email: mholland@nifa.usda.gov
Specific Aims: To oversee the mutagenesis, cDNA assembly and recovery of recombinant PEDV encoding a variety of mutations associated with altered virulence, either in natural isolates or in genetic targets (ExoN, nsp16 2-0 methyltransferase activity) shown to attenuate other recombinant CoV.

(b)(4) (PI: Baric) 06/23/16-06/22/18 (b)(4) mon
$1,066,500
Breadth of Blockade Antibody Responses Following Norovirus Vaccination
To conduct a project as an agreement in which Dr. Ralph Baric will test Takeda provided serum samples for cross-strain blockade antibody responses.
Contracting Officer: (b)(4)
Specific Aims: To conduct a project as an agreement in which Dr. Ralph Baric will test (b)(4) provided serum samples for cross-strain blockade antibody responses. This study is one in a series and thus experimental protocols, institutional approvals, and cost considerations are all cogent factors for entering this agreement.

R01AI089726 (PI: Li) 06/07/16-05/2831/18 (b)(4) mon
Univ Minn/NIH $120,384
Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis.
Role: Subcontract PI
Grants Management Specialist: Erik Stemmy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: erik.stemmy@nih.gov
Specific Aims: Aim 1 examines receptor binding by CoV S1-NTDs. Specifically, we will investigate whether S1-NTDs from different CoV genera have the same structural fold and evolutionary origin as host galectins (galactose-binding lectins). We will also examine how CoV S1-NTDs recognize sugar receptors. These studies will reveal the evolutionary origins of CoV S1-NTDs, enhance understanding of sugar recognition by CoVs, and may facilitate future design of sugar analogues and subunit vaccines to inhibit CoV infections. Aim 2 focuses on receptor binding by CoV S1-CTDs. Specifically, we will analyze the interactions between the S1-CTDs of bat SARS-like CoVs (SL-CoVs) and the protein receptor homologues from humans and other animals, and elucidate how bat SL-CoVs transmitted to humans and other animals to cause the SARS epidemic through evolutionary changes in their S1-CTDs. These studies will provide critical information for understanding emergence potential of bat SL-CoVs and for facilitating epidemic monitoring and control.

R01 AI127845 (Becker-Dreps) 09/27/16-08/31/21 (b)(4) mon
NIH $508,749
Natural history, immunity, and transmission patterns of sapovirus in a Nicaraguan birth cohort

The proposed study assembles a team of investigators with diverse strengths to elucidate the natural history, immunity, and transmission patterns of sapovirus in a birth cohort of 400 Nicaraguan infants. The overarching goal of this proposal is to provide needed data to inform prevention and control interventions, including future vaccine development.

Grants Management Specialist: Kelvin D. Lyons NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: kelvin.lyons@nih.gov

Specific Aims: Aim 1. Characterize the natural history and risk factors for sapovirus gastroenteritis in a birth cohort. Aim 2. Elucidate the development of immunity to sapovirus in early childhood and the potential protective effect of maternal immunity. Aim 3. Apply novel genetic and spatial analytic tools to epidemiological data generated in Aim 1 to characterize patterns of sapovirus transmission within households and communities.

(b)(4) (PI:Sims) 06/07/17-06/06/18 (b)(4)
$120,000

Testing (b)(4) Nucleoside Analog Compounds

Testing (b)(4) nucleoside analog compounds against highly pathogenic and zoonotic coronavirus strains in primary human airway epithelial cell (HAE) cultures has provided essential efficacy data for the preclinical development of GS-5734. As such, the Baric laboratory will perform additional compound testing in 2017.

Role: Investigator

Contracting Officer: (b)(4)

Specific Aims: These experiments will focus on two areas: 1) novel second generation compounds or compounds not previously provided by (b)(4) and 2) selecting and evaluating drug resistance profiles for SARS-CoV and MERS-CoV mutants in primary human lung cells.

R01 AI132178 (MPI: Baric/Sheahan) 08/09/17-07/31/22 (b)(4)
NIH $1,184,372

Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

The primary goal of our program is to accelerate the preclinical development of GS-5734 and promote IND licensure for the MERS-CoV indication. In parallel, we will assess efficacy against a panel of CoV representative of family-wide genetic diversity, including prepandemic zoonotic strains poised for emergence, to thoroughly evaluate the breadth of antiviral activity and predict efficacy against future emerging CoV.

Grants Management Specialist: Kelvin D. Lyons NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: kelvin.lyons@nih.gov

Specific Aims: In Aim 1, we refine the pharmacokinetics and pharmacodynamics of GS-5734 through the study of drug metabolism in various primary human lung cells targeted by CoV and evaluate in vivo efficacy in murine and non-human primate models of MERS- and SARS-CoV. In Aim 2, we define the breadth of efficacy and resistance profile through challenge with a diverse array of genetically distinct CoV, selection of resistance mutants against SARS- and MERS-CoV, and assessment of the effect of resistance on virus replication, fitness and susceptibility to treatment. In Aim 3, define the mechanism of action of GS-5734 via single molecule RNA fluorescence in situ hybridization and deep RNA-sequencing of vehicle or drug treated infected cells and mice revealing the effect of treatment on viral RNA and/or alteration of antiviral immunity. We articulate a development strategy for broad-spectrum therapeutics that could be extended to a multitude of emerging viral pathogens threatening global public health.

PENDING:

(b)(4)

To identify nonneutralizing antibodies which enhance ZIKV infection in primary adult and fetal cord monocytes (antibody dependent enhancement-ADE), which may be associated with more severe clinical presentations like Guillain-Barre syndrome and microcephaly.

Not Assigned (PI: Breuer) 02/01/17-01/31/20
(b)(4) University College London 500,000£
(b)(4)

Why do Norovirus pandemics occur and how can we control them?
The program uses hospital and community cohorts of NoV infected individuals to ask fundamental questions into the molecular and evolutionary epidemiology of human NoV infections, focusing on the GII.4 strains, leading to new models of virus emergence and disease prevention. (Funded pending execution of subcontract)

COMPLETED:
R01AI075297 (PI: Baric) 4/1/08 - 3/31/13
NIH-NIAID $385,247
SARS-CoV Pathogenic Mechanisms in Senescent Mice
Our central hypotheses are that determinants in the S glycoprotein promote severe disease by corrupting the role of antgiotensin 1 converting enzyme 2 (ACE2) in the prevention of acute lung injury and by the induction of innate immunity and complement. We will identify the genetic factors in the virus and host that regulate SARS-CoV pathogenesis. In aim 1, we study the pathogenesis of lethal and nonlethal SARS-CoV infection in young and senescent mice, determining if chronic disease manifestations of pulmonary fibrosis occur in survivors.
Grants Management Specialist: Erik Stemmy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: erik.stemmy@nih.gov
Specific Aims: These studies will simultaneously evaluate the effects of select alleles that evolved during the course of the outbreak on SARS-CoV replication and pathogenesis, providing insight into the genetic factors that contributed to the 2002-2003 epidemic.

HHSN272008000060C (PI: Katze) 9/15/08 – 9/30/13
Univ Wash/NIH $1,017,398
A Systems Biology Approach to Emerging Respiratory Viral Diseases,
Baric SubProject: Systems Biology of Lethal and Attenuated SARS-CoV Infection
Grants Management Specialist: Martin Gutierrez NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: mgutierrez@mail.nih.gov
Specific Aims: The overall hypothesis is that highly pathogenic respiratory viruses use common and unique strategies to mechanistically remodel the intracellular environment and signaling pathways of the host cell to enhance virus replication, regulate disease severity and promote virus transmission. Using SARS-CoV, H1N1 2009 and a comparative systems biology approach with highly pathogenic H5N1 avian influenza virus, we will identify unique and common signaling circuitry that is essential for promoting severe disease profiles in the lung.

R01 AI 056351 (PI: Baric) 04/15/09 – 03/31/14
NIH-NIAID $381,497
Susceptibility and Protective Immunity to Noroviruses
This application seeks to study the function of susceptibility alleles in human Norovirus infection. Using a human challenge model, we will determine if individuals initially infected with Norwalk virus develop long-term resistance that protects against subsequent challenge. We will also determine if other Noroviruses use ABH antigens as receptors for docking and entry.
Grants Management Specialist: Frederick Cassels NIAID, 5601 Fishers Lane MSC 9806 Bethesda, MC 20892 Email: casselsf@niaid.nih.gov
Specific Aims: This study investigates the molecular mechanisms governing norovirus capsid evolution, structure and HBGA recognition as a function of immune driven antigenic drift and deceptive imprinting. We identify the mechanisms by which replacement strains evolve over time, recognize new HBGA carbohydrate binding targets, and escape from highly penetrent host susceptibility alleles and protective herd immunity. While defining the relationships between mutation, antigenic variation, immunogenicity, deceptive imprinting, structure and HGBA

binding, our interdisciplinary team simultaneously develops a robust GII.4 challenge model in swine and characterizes human monoclonal antibodies (mAb) to GI and GII strains, providing key reagents for advancing the field.

U54 AI081680 (PI: Nelson) 03/01/09-02/28/14
Oregon Health Science Univ/NIH $427,722
Pacific Northwest Regional Center for Excellence in Biodefense and Emerging Infectious Diseases.
Defining Host and Viral Genetic Determinants of Viral Pathogenesis and Immunity
Project 3.1: Systems Pathogenomics of Acute Respiratory Virus Infection
Role: Subcontract PI
Grants Management Specialist: Laura C. Eisenman NIAID, 5601 Fishers Lane MSC 9806 Bethesda, MC 20892 Email: le55d@nih.gov
Specific Aims: Aim 1: Host genetic regulation of SARS-CoV and influenza infection. Aim 2: Systems genetics of age-related susceptibility to severe respiratory virus infection. Aim 3. Predictive Genetic Medicine. Aim 4: Systems Pathogenomics of Severe Respiratory Virus Infections. Aim 5: Regulation of Severe Lung Disease in Macaques.

U54-AI057157 (Sparling, Project 3.2 PI: Baric) 03/15/2009 – 02/28/2014
NIH-NIAID $6,566,370
SERCEB Southeast Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases - Project 3.2 Antibody in Protective and Pathogenic Immunity to Dengue Type 3
Grants Management Specialist: Michael Schaeffer NIAID, 5601 Fishers Lane MSC 9806 Bethesda, MC 20892 Email: mschaefer@niaid.nih.gov
Specific Aims: Making Dengue 3 recombinant viruses and isolating monoclonal antibody escape mutants. Project 1.1 Platforms for Synthesis and Testing of Emerging Zoonotic Viruses. Overall goal: Use the Category C SARS-CoV and zoonotic Bat-CoVs as a model system to develop and test platform approaches for rapid recovery and universal control of all known and future emerging CoVs.

R01AI085524 (PI: Marasco) 06/09/10-05/31/15
Dana Farber/NIH $175,723
Broad Spectrum Neutralizing Human Abs to SARS-CoV and Related Zoonotic Coronaviruses.
To use SARS-CoV as a model to: 1) establish new paradigms for developing universal therapeutic platforms that protect against new emerging and deliberately designed human pathogens; 2) define pathways of virus escape as a function therapeutic composition and evaluate pathogenic consequences and 3) evaluate therapeutic potential in robust animal models, especially in vulnerable populations that develop acute respiratory distress syndrome (ARDS).
Grants Management Specialist: Erik Stemmy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: erik.stemmy@nih.gov
Specific Aims: To develop platforms for rapidly identifying broad-spectrum neutralizing human mAbs (BnAbs) that are effective against emerging epidemic and zoonotic pool strains, using SARS-CoV as a model. Major strengths of this model include robust sequence database of epidemic and zoonotic isolates, synthetic genomics and reverse genetics and in vivo pathogenesis models in young and aged mice that recapitulate severe human end-stage lung disease and ARDS; atomic structures of SARS-CoV receptor ACE2 and human BnAbs bound to its Spike protein; and well developed methodologies and novel reagents developed by our collaborative team. Our approach is based on our published data that BnAbs can be designed to either prevent virus neutralization escape and/or attenuate virus evolution. Information gained from in vitro studies will be used to develop, in parallel, new strategies for maximizing vaccine efficacy against the broader heterogeneous pool of animal strains. The impact of these studies will be high, providing a template for similar design platforms for other important human emerging pathogens.
Role: Investigator

HHSN272201000191/HHSN27200001 (PI: Palese) 9/30/2011-9/29/2012

Mt Sinai School of Medicine/NIH/NIAID $200,771

NIAID Animal Models of Infectious Diseases-Task Order A26

New Animal Models for Chronic Chikungunya Virus Diseases in At-Risk Populations

The goal of this project is to test a variety of mouse strains for their susceptibility to chronic chikungunya virus-induced disease.

Role: Consortium PI

Grants Management Specialist: Maureen Beanan NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: beananm@mail.nih.gov

Specific Aims: The goal of this project is to test a variety of mouse strains for their susceptibility to chronic chikungunya virus-induced disease.

HHSN272201000019I-HHSN27200003 (PI: Palese) 09/30/11-03/31/17

MSSM/NIH $481,223

MERS-CoV Mouse Model for Vaccine and Therapeutic Testing (Task Order A57)

This contract provides for the development and standardization of small animal models for infectious diseases, and may include efficacy testing of candidate products.

Grants Management Specialist: Maureen Beanan

NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806

Email: beananm@mail.nih.gov

Specific Aims: Use generation of transgenic mice and modifications to the MERS-CoV genome to identify a mouse model for MERS-CoV that recapitulates human disease phenotypes for evaluating vaccine platforms and therapeutics.

684K644 Supplement to OMIC (PI: Kawaoka) 6/1/16-5/31/17

Univ. of Wisconsin/NIH/NIAID $200,000

Systems Virology for MERS-CoV in vivo

The goal is to develop systems biology datasets and unbiased modeling algorithms to deconvolute the complex pathogen-host interactions that regulate severe disease outcomes following infection and identify common host pathways/genes that can be exploited for therapeutic control. These studies will build on our current data set by collecting data sets for MERS-CoV in vivo.

Role: Investigator

Grants Management Specialist: Marciele M. Degrace NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: degracemm@mail.nih.gov

Specific Aim: Systems Biology of MERS-CoV Pathogenesis in vivo.

R56-AI097560 (PI: Desilva) 08/21/12-07/31/13

NIH/NIAID $225,000

Dissecting the Human Antibody Response to Dengue Virus

We propose to test the hypothesis that new epitopes created by the assembly and close packing of viral proteins on the surface of the virus are the main targets of the functionally important human antibody response. The impact of this work will be far ranging as it will redirect a field that has mainly focused on B-cell epitopes on subunits of E protein to consider new structural features and epitopes created following viral assembly.

Grants Management Specialist: Maggie Wells, NIAID, 6700B Rockledge Drive, Room 2250, MSC 7614 Bethesda, MD 20892-7614 Email: mw509s@nih.gov

Specific Aims: Aim 1: Identification of the fraction of antibodies in polyclonal dengue immune human sera responsible for potent virus neutralization. Aim 2: Identification of the fraction of antibodies in human immune sera responsible for antibody dependent enhancement of DENV.

Role: Investigator

OVERLAP:

If another application is funded, effort among the above projects will be adjusted such that the total effort does not exceed 100%.

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

Provide active support for all key personnel. **Other Support includes all financial resources, whether Federal, non-Federal, commercial or institutional, available in direct support of an individual's research endeavors, including but not limited to research grants, cooperative agreements, contracts, and/or institutional awards.** Training awards, prizes, or gifts do not need to be included.

There is no "form page" for other support. Information on other support should be provided in the *format* shown below, using continuation pages as necessary. ***Include the principal investigator's name at the top and number consecutively with the rest of the application.*** The sample below is intended to provide guidance regarding the type and extent of information requested.
For instructions and information pertaining to the use of and policy for other support, see Other Support in the PHS 398 Part III, Policies, Assurances, Definitions, and Other Information.
Note effort devoted to projects must now be measured using person months. Indicate calendar, academic, and/or summer months associated with each project.

Format

NAME OF INDIVIDUAL
ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

Samples

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT FOR ALL KEY PERSONNEL – OREGON HEALTH & SCIENCE UNIVERSITY

DEFILIPPIS, V

ACTIVE

HHSN272201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
Adjuvant Discovery Program $1,787,755
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity
The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: Co-Investigator / Project Lead on awarded supplement

Global Health Proposal OPP1107409 (Picker) 7/28/2014 – 8/31/2019 (b)(4)
Bill & Melinda Gates Foundation $6,144,126
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Leader

(THIS AWARD)
5 U19 AI109680-04 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $299,955
Antiviral Drug Discovery and Development Center
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project 3B Co-Investigator

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

INACTIVE

5 U54 AI081680-05 (Nelson) 3/01/2011 – 2/28/2015 0.0 calendar
NIH/NIAID (no cost extension)
Pacific NorthWest Regional Center of Excellence Developmental Research Project
Role of cytokines in Chikungunya virus-associated disease
The goal of this project is an understanding of the contribution of these host-virus interactions to CHIKV-associated disease. CHIKV is a re-emerging arthritogenic mosquito-borne RNA Alphavirus. Infection results in serum induction of multiple proinflammatory cytokines including type I interferons (IFN).
Role: Developmental Research Project (DP005) PI

OVERLAP No overlap

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT

DIAMOND, MICHAEL

ACTIVE

R01AI104972 (Diamond/Chanda) 04/01/13-03/31/18
NIH/NIAID $278,755
Role: PI
(b)(4)

ISG Control of Flavivirus Infection

The continual outbreaks of flavivirus disease highlight a need for an expanded understanding of mechanisms of immune control. Insight into the cell-intrinsic immune processes that restrict flavivirus infection is essential for developing novel strategies to contain disease. The studies in this collaborative and inter-disciplinary project between the Diamond and Chanda laboratories will use genetic screens to identify novel interferon stimulated genes (ISG) that modulate flavivirus infection in specific cell types ex vivo and in vivo.

U19AI106772 (Kawaoka) 07/01/13-06/30/18
NIH/NIAID $304,060
Role: Co-Investigator
(b)(4)

Modeling Host Responses to Understand Severe Human Virus Infections

Research in this program project grant will depend on unique cell and tissue reagents generated by the Diamond laboratory at Washington University. These reagents will be used to perform global transcriptome, proteomics, metabolomics, and lipidomic analysis by the Project Core facilities. Target genes will be identified by network analysis and the Diamond laboratory will validate these using reverse genetic approaches. In the last Aim, new KO mice will be generated to assess the physiological relevance of target genes in restricting West Nile virus infection in vivo.

R01AI073755 (Diamond) 06/01/13-05/31/18
NIH/NIAID $546,211
Role: PI
(b)(4)

Antibody Based Protection against Dengue Virus

This is a collaborative research project with the goal of defining new states of DENV particle structure and determining how these interact with specific MAbs. This information will be translated into developing a novel DENV vaccine strategy that traps virions in states that preferentially elicit highly neutralizing Abs.

R01AI104002 (Gale) 07/01/13-06/30/18
NIH/NIAID $221,776
Role: Co-PI
(b)(4)

Innate Immune Control of West Nile Virus

The Diamond laboratory will perform a series of interactive studies aimed at understanding the mechanistic contribution of RLR signaling, specific IFN-β defenses restricting neuronal cell tropism, and viral counter-measures in determining WNV infection outcome. We hypothesize that WNV infection outcome is regulated by virus/host interactions that modulate RLR signaling and specific IFN-β innate immune defenses in distinct cell types.

HHSN272201400018C(Fremont) 09/30/14-09/29/18
NIH/NIAID $275,067
Role:Co-Investigator
(b)(4)

B-Cell Epitope Mapping of Viral and Parasitic Antigenes

The primary goal is the delineation of epitopes recognized by potently neutralizing antibodies and establishing correlates of protection that can aid in the development of vaccines and therapeutics against human pathogens

U19AI083019 (Gale) 05/01/14–04/30/19
NIH/NIAID $220,741
Role: Co-Investigator
(b)(4)

Center for the Study of Immune Mechanisms of Flavivirus Control
Project 2: CNS Innate Immune Control of Encephalitic Flaviviruses
The primary goal of Project 2 is to determine how innate immune responses impact on the entry, infection, and replication of encephalitic flaviviruses within the central nervous system (CNS). Our experiments will assess the virus and host interface that regulates the innate immune response and controls WNV pathogenesis in the central nervous system, which will reveal novel targets for therapeutic development to suppress flavivirus infection and minimize neuronal injury.

HDTRA1-15-1-0013 (Diamond) 07/01/14-06/30/19 (b)(4)
DTRA $154,558
Role: PI
A novel inactivated trivalent vaccine to prevent infection by Venezuelan, Eastern, and Western equine encephalitis viruses
The goal of this project is to determine the potency of Ab neutralization, extent of cross-neutralization against VEEV, EEEV,and WEEV, and location of key neutralizing epitopes on newly generated panels of monoclonal antibodies (MAbs). This project will elucidate the immunology and biology of encephalitic alphaviruses and generate an effective counter-measure to protect American citizens and military personnel against infection.

R01AI114816 (Crowe /Diamond) 02/01/15-01/31/20 (b)(4)
NIH $176,219
Role: PI
Structural and functional basis of ultra-potent CHKV neutralization by human mAbs
A primary goal of this project is to define the molecular, genetic, immunologic, and structural characteristics of ultra-potent neutralizing human mAbs with broad activity against all genotypes of CHIKV. Additional goals include defining the mechanistic correlates of protection by these ultra-potent neutralizing mAbs. In these studies, we will elucidate how antiviral Abs with exceptional inhibitory activity exert their action in cell culture and in vivo.

P01AI106695 (Hariss) 07/01/15-6/30/20 (b)(4)
NIH $100,000
Protective Immunity Following Dengue Virus Natural Infections and Vaccination
Role: Co-Investigator
The Diamond laboratory will perform the initial screens of virus specificity and neutralization potentialof the monoclonal antibodies generated from peripheral blood mononuclear cells (PBMCs) after natural DENV infection or immunization with tetravalent vaccines (obtained from Core C). They will also generate the labeled viruses needed for the DENV-specific sorting of memory B cells by the Crowe laboratory for one of the methods to be used in Core B for generating MAbs for Projects 1 and 2.

R01 AI125202 (Sekaly) 06/01/16-05/31/21 (b)(4)
NIH $154,738
Role: Co-Investigator
A multi-tiered approach to develop validated assays to predict efficacy of a tetravalent live attenuated Dengue Virus vaccine in Phase II and Phase III clinical trials
Testing of new assays for serum/plasma neutralization of DENGUE (DENV) vaccine samples to be evaluated by the Diamond laboratories.
Role: Co-Investigator

R01AI123348(Dermody/Diamond/Morrison) 06/01/16-05/31/21 (b)(4)
NIH $169,892
Role: Co-PI
Chikungunya Virus Cell Entry and Pathogenesis
The goal of the project is to test the hypothesis that chikungunya virus (CHIKV) requires specific host glycosaminoglycans (GAGs) and COPI transport components for cell tropism and disease pathogenesis.

UH2NS100126 (Klein/Diamond) 09/30/16-06/30/18 (b)(4)
NIH/NINDS $49,060
Role: Co-PI
Effect of Aging on Neuroinvasion During West Nile Virus (WNV) Infection
In this proposal, we will examine the impact of age on innate and adaptive immune mechanisms that control viral entry and clearance specifically within the CNS. The UH2 phase we will establish breeding cohorts and determine the feasibility of using 21 month-old aged animals in WNV infection studies. In the UH3 phase, we will explore how changes in skin immunity, BBB integrity, and CNS inflammation during aging influence neuroinvasion and disease progression associated with WNV infection.

R01AI127774(Gack**)** 09/01/16-8/31/21 (b)(4)
NIH $142,447
Flavivirus NS3-Mediated Immune Escape
The experimental approach of this section by the Diamond laboratory will use WT and *Mavs*-/- mice infected with WT or isogenic NS3 mutant WNV strains to perform viral burden and survival analyses, cell type-specific viral growth kinetics, and analyses of cell intrinsic and extrinsic immunity. Overall, the proposed work may have implications for understanding how flaviviruses attenuate early innate immune responses, which could provide novel targets for pharmacological inhibition or for rational attenuation and vaccine strain development

R21-AI128090-01 (Lai/ Diamond) 01/01/18-12/31/19 **0.00 cm**
NIH $92,324 (effort starts in year 2)
Role: Co-PI
Engineered Dengue EDIIIs as Broad Immunogens Goals: To engineer and test novel Dengue vaccine candidates
The overarching goal of this work is to use innovative protein engineering approaches to overcome the traditional barriers with EDIII-based immunogens. This work will provide proof-of-concept in mice for novel subunit vaccine candidates against DENV, and possibly other flaviviruses of global concern.

R01AI127828 (Crowe, Diamond) 9/01/16-8/31/17(21) (b)(4)
NIH $196,647
Role: Co-PI
Human neutralizing antibodies for Zika virus
The primary goal of this project is to define the molecular, genetic, immunologic, characteristics of newly-isolated neutralizing human mAbs with broad specificity against all strains of ZIKV. A second goal is to define the mechanistic correlates of protection by neutralizing mAbs. A third goal is to determine whether cross-reactive anti-DENV human MAbs that bind to ZIKV are protective/therapeutic or pathogenic in a newly developed mouse model of ZIKV

R01HD091218 (Mysorekar/Diamond) NEW 4/1/17-3/31/18(22) (b)(4)
NIH / NICHD $207,143
Role: Co-PI
Mechanisms of Zika Virus Maternal-Fetal Transmission
The goal of this proposal is to identify how ZIKV is able to cross the placental barrier to cause adverse outcomes in the baby. This work has significant clinical implications for understanding ZIKV-pathology and for developing strategies for mitigating ZIKV trafficking through the placenta and thus reducing adverse outcomes.

R21AI131254 (Curiel) NEW 4/1/17-3/31/18 (19) (b)(4)
NIH $62,499
Role: Co-Investigator
Gorilla Adenovirus Zika Vaccine for Humans
The goal of this proposal is to design novel ZIKV vaccine candidates and conduct initial preclinical testing in newly-generated mouse models of infection and disease

(b)(4)

R01AI132186 (Slifka/Diamond) NEW 6/1/17-5/30/18(22)
NIH/OHSU $159,635

An advanced Vaccine Candidate to Prevent Zika Virus Infection

The role of the Diamond laboratory in this project will be to characterize the breadth and magnitude of the neutralizing antibody response against Zika virus and to perform the critical challenge studies using newly developed mouse models of infection. In addition, the Diamond laboratory will help to assess safety of the vaccines by performing challenge studies with related flaviviruses. These studies comprehensively will establish the functional quality of antibodies generated by the candidate vaccine.

(b)(4)

HHSN272201400018C(Fremont) 7/1/17-6/30/18(19)
NIH/NIAID $422,729

B Cell Epitope Mapping of Viral and Parasitic Antigens

The objective of this proposed supplement is to precisely define B-cell epitopes for ZIKV glycoproteins and develop correlates of protection or pathogenesis.
Role: PI

(b)(4)

1R01AI125618-01A1 (Artomov) NEW 08/1/17-07/31/18 (22)
NIH/NIAID $250,000

Itaconate as metabolic regulator of inflammation

Metabolic rewiring is important regulatory mechanism controlling activation of the immune cells. Succinate is one of the major metabolic regulators of macrophage activation, playing distinct proinflammatory role. Our preliminary data suggest that high production of itaconate during macrophage activation is functionally critical, as it provides a natural, structural mimetic metabolic regulator to balance the pro-inflammatory function of succinate. In this grant, we plan to decipher the regulatory role of itaconate and determine its functional impact on immune responses in vivo and in vitro.
Role: Co-Investigator

(b)(4)

R01AI101400 (Lemke) NEW 12/01/16 – 11/30/2022
NIH/NIAID $323,296

TAM Receptors and Flavivirus Infection

This grant studies how TAM receptors (Axl, Mer, and Tyro3) contribute to flavivirus pathogenesis. These molecules are now believed to enhance infection of West Nile and Dengue virus. Our lab will perform in vivo experiments in KO mice to determine the exact contribution of these molecules to pathogenesis.
The focus of the Klein laboratory part of this collaborative grant is to determine how cell-type specific functions of TAM receptors modulate West Nile virus (WNV) neuropathogenesis and recovery. We will determine the effects of cell-type specific deletion of Mer and Axl on viral sensing at the blood-brain barrier (BBB), identifying cellular constituents of the BBB that determine permeability to virus and antiviral leukocytes. Using a novel model of recovery from WNV we will also determine how global Axl and Mer deletion or their expression by microglia and astrocytes contribute to the development of cognitive deficits during recovery from WNV encephalitis via analysis of neurogenesis, synapse elimination and spatial learning. We will also evaluate the effects of cell-type specific deletion of Mer and Axl on BBB permeability and viral crossing during La Crosse and Venezuelan equine encephalitis virus pathogenesis.
Role: Co- Investigator

(b)(4)

R01AI127513 (Lenschow/Diamond) NEW 7/01/17-6/30/22
NIH $370,640 ($135,572 Diamond Lab)

IFN Independent Control of Acute and Chronic Chikungunya Virus Induced Disease

Chikungunya virus is a mosquito-transmitted alphavirus that causes persistent, debilitating polyarthritis in a significant numbers of patients. The host generates a robust immune response during infection, including the up-regulation of type I and type III interferons. Our goal is to understand how these different interferons regulate viral replication and host immunity during acute and chronic CHIKV infection, and whether augmenting or blocking these pathways will affect disease outcomes.
Role: PI

OVERLAP:
None

Expired project since last reporting period:

R01AI098715 (Rico-Hesse) 05/01/12 – 05/31/17 (b)(4)
NIH/NIAID $65,837
Role: Co-Investigator

Therapy of Dengue with Modified Antibodies in Humanized Mice

The foundation of this proposal is the existing collaboration between an academic (Diamond) laboratory with broad expertise in flavivirus pathogenesis and antibody neutralization, and a company (MacroGenics) with skill in humanization of mouse antibodies as therapeutic agents.
These collaborators have produced humanized mAbs against flaviviruses with optimized effector function and no possibility of antibody-dependent enhancement of infection. The Rico-Hesse laboratory brings experience in dengue virus pathogenesis, with emphasis on the use of wild-type viruses to test determinants of virulence and transmission. Dr. Rico-Hesse serves as the P.I. in this collaboration because most of the experiments described here will involve the use of the mouse model of disease developed in her laboratory. Thus, we propose an experienced collaborative group to test novel dengue virus immunotherapy in a more relevant 0.small animal model of disease.

R33AI101329 (Chen) 06/01/12-05/31/17 (b)(4)
NIH/NIAID $144,106
Role: Co-Investigator

Bi-Functional Antibodies with Targeted CNS Delivery Against West Nile Virus

The Diamond laboratory will characterize the antiviral and immunological activity of different cell culture and plant derived bi-functional antibodies using mouse TfR-specific hE16 bifunctional antibody as a proof-of-principle in a mouse model of WNV infection. Our lab will test the hypothesis that TfR-Bif can achieve higher levels in the CNS and extends the window of treatment against WNV encephalitis.

BAA-NIAID-DAIT (Dorenz) 01/01-16-12/31/17 (b)(4)
HHSN2722014010058C $65,690
Role: Co-Investigator

B-cell Epitope Discovery and Mechanisms of Antibody Protection for HCV, RABV, and EBOV Envelope Proteins

The primary objectives of this U19 program are to use mathematical modeling to define factors that contribute to and predict severe dengue-related illness, to identify biomarkers of disease severity, and validate their function in cell culture and animal models of dengue pathogenesis. CA refined understanding of dengue pathogenesis almost certainly would lead to more rapid diagnosis, improved case management, and identification of new therapeutic and vaccine strategies. We hypothesize that multiple pathways of immune activation and dysregulation, mediated by both the innate and adaptive immune system, contribute to dengue pathogenesis and disease. We believe that an integrative approach that considers these immune perturbations together in a mathematical model will be essential to fully understand the mechanisms of disease. The Atkinson laboratory will analyze the role of complement in Dengue pathogenesis in humans using genetic and biochemical approaches, and the Diamond laboratory will use a new more immunocompetent mouse model of Dengue infection to assess the role of key innate immune modulators.

OTHER SUPPORT

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

PHS 398/2590 OTHER SUPPORT

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

HIRSCH, A

ACTIVE

(THIS AWARD)
5 U19 AI109680-04 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $337,039
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates

The overall goal of this proposal is to discover and characterize compounds with broad anti-flavivirus activity. Role: Co-Investigator

HHSN27201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
NIH/NIAID $1,787,755
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity

The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses. Role: Co-Investigator

1 R21 HD091032-02 (Streblow) 9/01/2016 – 8/31/2018 (b)(4)
NIH $150,000
Development of a NHP Model for Determining the Causal Relationship Between Zika Virus Infection During Pregnancy and Fetal Microcephaly

The goal of this project is to develop a NHP model of Zika virus infection and fetal disease.
Role: Co-Investigator

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

OVERLAP

No active or pending application has scientific or budgetary overlap. If a pending grant is awarded, effort will be adjusted following NIH guidelines.

INACTIVE

BAA-NIAID-DAIT-HIHAI2010085 (Nikolich-Zugich PI) 5/16/2011 – 5/15/2016 (b)(4)
Subcontract Y562709 on NIH Contract No. HHSN272201100017C
NIH/NIAID $195,051
Protective Immunity in Special Populations: Interface between Innate and Adaptive Immunity

The major goal of this subcontract is to determine the role of miRNAs on immune function in the context of aging. Role: Co-Investigator

5 R21 AI101282-02 (Hirsch) 6/01/2012 – 5/31/2015 (b)(4)
NIH/NIAID $120,846 (no-cost extension)
Evaluation of Host miRNAs as Therapeutics against Encephalitogenic Flaviviruses

The overall goal of this proposal is to use cellular microRNAs (miRNAs) as potential targets of therapeutic intervention for the neurotropic flaviviruses West Nile virus (WNV) and Japanese encephalitis virus (JEV).
Role: PI

5 U54 AI 081680-05 (Nelson) 4/20/2009 – 2/28/2015 0.00 calendar
NIH/NIAID $5,688,792 (no-cost extension)
Pacific Northwest Regional Center of Excellence – Developmental Project: The role of microRNAs in flavivirus replication (DP 006)

The overall goal of this proposal is to elucidate how the observed changes in miRNA expression affect WNV replication and pathogenesis as well as to extend this analysis to the dengue viruses, which are also members of the flavivirus family. Role: PI of Developmental Project

OTHER SUPPORT

MORRISON, T.

ACTIVE

U19 AI109680 (Whitley) 3/1/2014 – 2/28/2019 (b)(4)
NIH/NIAID $149,913
Title: Antiviral Drug Discovery and Development Center
The major goals of this project are to i) identify small molecules capable of inhibiting replication of diverse members of the *Alphavirus* genus and ii) test candidate molecules for prophylactic and therapeutic efficacy against chronic chikungunya (CHIKV) infection and joint disease.

R01 AI108725 (Morrison) 7/1/2014 – 6/30/2019 (b)(4)
NIH/NIAID $250,000
Title: Mechanisms of Immune Suppression During Arthritogenic Alphavirus Infections
The major goals of this project are to i) define mechanisms by which iNOS and Arg1-mediated production of peroxynitrite suppresses T cell responses to infection and ii) to define cytokine signaling pathways that promote the immunosuppressive activity of macrophages and impair control of Ross River virus infection in musculoskeletal tissues.

R01 AI123348 (Dermody, Morrison, Diamond) 4/1/2016 – 3/31/21 (b)(4)
NIH/NIAID $114,301
Title: Chikungunya Virus Replication and Pathogenesis
The major goals of this project are to i) determine how CHIKV E2 engages specific GAGs and the role of GAG binding in CHIKV pathogenesis, ii) discover mechanisms by which COPI transport promotes CHIKV infection, and iii) identify the cell types infected by CHIKV in mice that contribute to acute and chronic disease.

PENDING
None

OVERLAP
None

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

NELSON, JA

ACTIVE

(NEW)
1 U19 AI128741-01 (Picker) 3/02/2017 – 2/28/2022
NIH/NIAID $178,259
(b)(4)
Development of Immunogenicity- and Efficacy-Optimized CMV Vectors for an HIV/AIDS Vaccine: Project 2: Optimization of CMV Vector CD8+ T Cell Response Programming by Modification of Vector Tropism

In this project, we seek to define the role of vector tropism in CMV vector CD8+ T cell response programming, and to exploit this biology to focus vector immunogenicity on the most efficacious response type. We will 1) determine how *in vivo* tropism of CMV vectors that differentially induce conventionally (MHC-Ia) restricted vs. unconventionally (MHC-E- and MHC-II-) restricted CD8+ T cell responses differ in infected tissues, 2) define the target cell tropism requirements for CMV vector-elicited MHC-E- and MHC-II-restricted CD8+ T cell responses using cell type-specific miRs, and 3) develop CMV vectors that preferentially or exclusively elicit MHC-E- and MHC-II-restricted CD8+ T cell responses by strategic modification of vector tropism.
Role: PI, Project 2

(NEW)
1 P01 AI127335-01 (Nelson/Yurochko) 8/15/2017 – 7/31/2022
NIH/NIAID $1,324,597
(b)(4)
Human Cytomegalovirus Dysregulation of Host Hematopoietic Progenitor Cell Signaling Pathways to Modulate Latency, Reactivation and Hematopoiesis during Transplantation

The goal of this PPG is to define Human Cytomegalovirus (HCMV) dependent signaling mechanisms in CD34+ human progenitor cells (HPCs) that regulate viral latency and reactivation as well as hematopoietic dysfunction using an *in vitro* HPC model, a humanized mouse model, and samples from Solid Organ and Hematopoietic Stem Cell Transplant Patients.
Role: PD/PI, Project and Core Leader

5 R01 AI021640-32 (Nelson) 12/01/1984 – 1/31/2018
NIH/NIAID $299,774
(b)(4)
Molecular Aspects of Cytomegalovirus Latency

The long-term goal of this project is to develop an understanding of the cellular and molecular mechanisms of human cytomegalovirus (HCMV) persistence in the host. This project will use HCMV miRNA mutants as well as miRNA inhibitory molecules in an in vitro CD34+ human progenitor cell system and a humanized mouse model to examine the role of the viral miRNAs in latency and reactivation.
Role: PI

HHSN272201400055C (Nelson) 9/30/2014 – 9/29/2019
NIH/NIAID $1,622,823
(b)(4)
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity

The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: PI

5 R01 AI120619-02 (Britt/Nelson) 4/01/2016 – 3/31/2021
NIH/NIAID $250,797
(b)(4)
HCMV miRNA Regulation of Secretion and Formation of the Viral Assembly Compartment

The major goals of this project are to elucidate the mechanisms through which human cytomegalovirus (HCMV) microRNAs (miRNAs) restructure the endocytic/secretory pathway to regulate secretion of cytokines, recycle proteins from the cell surface and form the viral assembly compartment (VAC).
Role: Co-PI (Multiple PI submission)

(b)(4) (Picker) 7/28/2014 – 8/31/2020 (b)(4)
$6,048,962
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (comprised of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Leader; Outcomes 2.2 and 2.3

(THIS AWARD)
5 U19 AI109680-04 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $274,539
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates
This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.
Role: Site PI; Project 1

8 P51 OD011092-57 (Robertson) 5/01/2014 - 4/30/2019 (b)(4)
NIH/OD (salary support only)
Support for Oregon National Primate Research Center
Role: Senior Scientist, Division of Pathobiology and Immunology

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

INACTIVE
5 R01 CA179921-02 (Moses) 5/01/2015 – 4/30/2020 (b)(4)
NIH/NCI $228,750
Heme Oxygenase-1 as a Tumor Factor and Therapeutic Target for Kaposi Sarcoma
The major goals of this project are to characterize the role of the host enzyme heme oxygenase-1 (HO-1) in KSHV pathogenesis and Kaposi sarcoma (KS), and to determine if HO-1 is a valid therapeutic target for KS.
Role: Co-Investigator

5 P01 AI094417-05 (Picker) 7/15/2011 – 6/30/2018 (b)(4)
NIH/NIAID (no-cost extension)
Development of an Effector-Memory T Cell AIDS Vaccine (Project 2: Attenuation of CMV Vector Pathogenicity and Transmission by Altering Viral Tropism)
The goal of this project is to determine whether genetically modifying CMV to limit its ability to replicate in cell types associated with disease and transmission, while retaining its ability to persist in cells important for eliciting immunity, will lead to a safe and effective vector for an HIV/AIDS vaccine. In this Program, we will modify CMV vectors and/or use complementary heterologous vaccines with CMV vectors to both increase the potency of CMV/SIV vectors so as to achieve rates of protection closer to 100% of vaccines, and reduce the pathogenicity and shedding potential of CMV vectors (while retaining immunogenicity), so as to achieve an effective vaccine that is safe enough for use in a general human population.
Role: Project 2 PI

NAME OF INDIVIDUAL: Ashish Kumar Pathak

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are…	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

ACTIVE –

Whitley (PI) 03/01/2014 – 02/28/2019 (b)(4)

NIH/NIAID 1U19AI109680-01 $1,025,151

UAB 000502793-011

Antiviral Drug Discovery and Development Center

The herein proposed Center of Excellence for Translational Research (CETR), which will be named the Antiviral Drug Discovery and Development Center (AD3C) has, at its center, the theme to develop new small molecule therapeutics for emerging and re-emerging viral infections. Translational research will focus on the inhibition of viral replication, especially viral polymerase.

Role: Core Leader, Medicinal Chemistry Core, Senior Medicinal Chemist

PENDING –

(b)(4)

OVERLAP – None.

OMB No. 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED

PHS 398 OTHER SUPPORT

PRICHARD, MARK PhD

ACTIVE

HHSN272201100016I (Prichard) 6/01/2011 – 05/31/2018
NIH/NIAID (Base Contract)
The goals of this contract are to evaluate compounds against human DNA viruses

HHSN27200014 (Prichard) 9/16/17– 9/15/18 (b)(4) months (b)(4)
NIH/NIAID TOR B27: In Vitro Assessment for Antimicrobial Activity $670,950
The goal of this contract is to evaluate compounds against human DNA viruses

HHSN272201100034C (Whitley 1) 9/28/11-08/15/2020 (b)(4) months (b)(4)
NIH/NIAID $5,894,081
Adaptive sequential study evaluating prevention of neonatal HSV: Detection of maternal shedding.

HHSN272201100035C (Whitley 2) 9/28/11-08/15/2020 (b)(4) months (b)(4)
NIH/NIAID $4,929,026
A Phase II 6 Weeks Oral Valganciclovir versus Placebo in Infants with Congenital CMV Infection.

HHSN272201100036C (Whitley 3) 9/28/11-08/15/2020 (b)(4) months (b)(4)
NIH/NIAID $4,306,108
Safety Tolerability and Pharmacokinetics of CMX001 in Renal Transplant Recipients with BKV.

HHSN272201100037C (Whitley 4) 9/28/11-08/15/2020 (b)(4) months (b)(4)
NIH/NIAID $3,996,088
A Pharmacokinetic/Pharmacodynamic and Resistance Evaluation of Intravenous Ganciclovir in Infants.

HHSN272201100038C (Whitley 5) 9/28/11-08/15/2020 (b)(4) months (b)(4)
NIH/NIAID $4,412,460
Adaptive study of CMX001 in infants with neonatal herpes simplex virus (HSV)

2R44AI100401-03 SBIR Phase 2 12/1/14-11/30/17 (b)(4) months (b)(4)
(TSRL subcontract)/NIH/NIAID $147,933
The goal of the research is to evaluate broad spectrum antiviral drugs against the DNA viruses

(THIS GRANT)
5U19AI 109680-05 (Whitley 6) 03/01/2014-02/28/19 (b)(4) months (b)(4)
NIH/NIAID $34,138,453
The goal of the research is to support influenza studies for CETR Grant

(b)(4) 8/22/16-12/21/17 (b)(4) months (b)(4)
Evaluation (b)(4) Compounds for Antiviral Activity $100,000

OVERLAP None

COMPLETED
HHSN2722010000271 (Quenelle) 8/31/16– 8/30/17 (b)(4) months (b)(4)
NIH/NIAID $606,340
Animal models for herpes simplex virus and human cytomegalovirus

OTHER SUPPORT

SHEAHAN, TIMOTHY

ACTIVE:

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4)
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator
Grants Management Specialist: Maureen BeananNIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: beananm@mail.nih.gov
Specific Aims Project 2: 1. To identify and develop inhibitors of CoV high-fidelity replication. 2. To identify and develop inhibitors of CoV RNA capping activity. 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors.

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator
Grants Management Specialist: Tina Parker NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: parkerti@mail.nih.gov
Specific Aims Project 2: In Aim 1, we will develop platforms to recover novel respiratory viruses and identify diagnostic and prognostic indicators of severe lung disease by infecting primary human lung and immune cells. In Aim 2, we use the Collaborative Cross Mice to develop new animal models of human disease and to define conserved genomic signatures that correlate with etiology and disease severity and then validate the role of these biomarkers in models of outbred human populations infected with different high and low path respiratory viruses. In Aim 3, the goal is to use structure-guided molecular diagnostics to identify key diagnostic neutralizing epitopes that inform susceptibility, epidemic potential and vaccine performance.

(b)(4) (PI: Sims) 06/07/17-06/06/18 (b)(4)
$120,000
Testing(b)(4) Nucleoside Analog Compounds
The overall goal of this project is to tes(b)(4) protease inhibitor/interferon cocktails in comparison to and with nucleoside analog compounds to determine the best course of treatment for patients infected with highly pathogenic human coronaviruses.
Role: Investigator
Contracting Officer: (b)(4)
Specific Aims: Aim 1. Determine EC50/CC50 values for interferon beta/Ritonavir/Lopinavir/GS-5734 treatment of MERS-CoV infected Calu3 cells. Aim 2. Determine EC50/CC50 values for interferon beta/Ritonavir/Lopinavir/GS-5734 treatment of MERS-CoV infected DPP4/Ces-/- mice.

R01 AI131688-01 (PI: Rice) 04/01/17-03/31/22 (b)(4)
Rockefeller/NIH
Analysis of immunity, viral adaptation and pathogenesis in a new mouse model of HCV-related rodent hepacivirus infection

Mechanisms that contribute to the persistence of hepatotropic viruses, such as HCV, are not well understood. We have recently established the first immune-competent mouse model of an HCV-related virus. With this new model, we propose to systematically study immunity and host-virus interactions during a hepatotropic RNA virus infection in vivo.
Role: Co-Investigator
Grants Management Specialist: Rajen Koshy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: rkoshy@niaid.nih.gov
Specific Aims: Access to human liver tissue is limited. The only immunocompetent animal model of HCV infection, the chimpanzee, is no longer readily available for research. However, we have recently succeeded in establishing the first immune- competent mouse model of an HCV-related virus, Norway rat hepacivirus (NrHV). Our preliminary characterization of this model revealed significant virological and immunological similarities with HCV infection in humans. This advance now opens the opportunity to interrogate hepatic antiviral immunity, host-virus interactions, viral adaptation, immune evasion strategies and pathogenesis of a hepatotropic virus at an unprecedented level. In this proposal we plan to comprehensively analyze innate and adaptive intrahepatic immune responses during hepacivirus infection in vivo and to define determinants of viral clearance.

1R01 AI132178-01 (MPI: Sheahan/Baric) 08/06/17-07/31/22 (b)(4)
NIH $1,184,372
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
In partnership with Gilead Sciences, we aim to accelerate the preclinical development of GS-5734 and promote IND licensure. We define the pharmacokinetics, pharmacodynamics, resistance profile, efficacy breadth and mechanism of action of GS-5734 against MERS-CoV and related emerging CoV.
Grants Management Specialist: Erik Stemmy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: erik.stemmy@nih.gov
Specific Aims: In Aim 1, we refine the pharmacokinetics, pharmacodynamics and breadth of GS-5734 through efficacy and metabolism studies in various primary human cells with a diverse array of human and zoonotic CoV and through the evaluation of in vivo efficacy in murine and non-human primate models of MERS- and SARS-CoV. In Aim 2, we select for resistance against SARS-CoV and MERS- CoV, and determine the effect of resistance on virus replication, fitness and susceptibility to treatment. In Aim 3, we determine if the mechanism of action of GS-5734 is a result of direct effects on viral RNA replication and/or alteration of antiviral immunity via deep sequencing and single molecule RNA fluorescence in situ hybridization of vehicle or drug treated infected cells and mice.

OVERLAP:
If another application is funded, effort among the above projects will be adjusted such that the total effort does not exceed 100%.

OTHER SUPPORT

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

(b)(4); (b)(6); (b)(3):7 U.S.C. § 8401

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

STREBLOW, D

ACTIVE

1 U19 AI128741-01 (Picker) 3/02/2017 – 2/28/2022 (b)(4)
NIH/NIAID $176,784
Development of Immunogenicity- and Efficacy-Optimized CMV Vectors for an HIV/AIDS Vaccine: Project 4: Optimization of CMV Vector CD8+ T Cell Response Programming by Modification of CMV-Encoded Molecular Mechanisms That Interfere with Unconventional CD8+ T Cell Response Priming
In Project 4 we first seek to identify the specific motifs in the UL128 and UL130 proteins which block MHC-E-restricted CD8+ T cell priming with the goal of mutating these regions to abrogate their MHC-E-response inhibition while retaining PRC function. Since MHC-E-restricted response priming is myeloid cell-dependent, whereas MHC-II responses are disfavored by vector infection of myeloid cells, this should result in a vector that preferentially elicits MHC-E-restricted responses. Second, since HCMV lacking UL128 and UL130 fails to elicit unconventional T cell responses in RM, it is possible that HCMV encodes other genes that block unconventional CD8+ T cell responses. Therefore, utilizing a deletion strategy we will ascertain whether any non-essential HCMV genes have this activity. Finally, utilizing the data from Projects 2-4, Project 4 will construct the 2nd generation “response and safety” optimized HCMV/HIV vector for manufacture and clinical testing in Project 5.
Role: PI, Project 4

5 R01 AI116633-02 (Streblow) 3/01/2016 – 2/28/2021 (b)(4)
NIH/NIAID $250,000
Characterizing the Role of CMV Latency in Solid Organ Transplant Rejection
The main goal of this project is to determine the mechanisms of cytomegalovirus-mediated solid organ transplant rejection.
Role: PI

(THIS AWARD)
5 U19 AI109680-04 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $299,200
Antiviral Drug Discovery and Development Center
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project Leader; Project 3B

(NEW)
1 P01 AI127335-01 (Nelson/Yurochko) 8/15/2017 – 7/31/2022 (b)(4)
NIH/NIAID $166,278
Human Cytomegalovirus Dysregulation of Host Hematopoietic Progenitor Cell Signaling Pathways to Modulate Latency, Reactivation and Hematopoiesis during Transplantation: Project 3: HCMV US28 regulation of host cell signaling in viral latency and hematopoiesis
In this project we will examine UL7 signaling in CD34+ HPCs and identify the cellular pathways necessary for viral reactivation and hematopoiesis in the context of infection. We will also determine whether these UL7 pathways cross-talk with pathways modulated by UL133/8 (Project 1), HCMV miRNAs (Project 2), US28 (Project 3) and wild-type HCMV infection (Project 5).
Role: Project Leader, Project 3

(b)(4) (Picker) 7/28/2014 – 8/31/2020 (b)(4)
$6,048,962
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Manager; Outcome 5 and Outcome 8

5 R21 HD091032-02 (Streblow) 9/20/2016 – 8/31/2018 (b)(4)
NIH $125,000
Development of a NHP Model for Determining the Causal Relationship between Zika Virus Infection during Pregnancy and Fetal Microcephaly
The goal of this project is to develop a NHP model of Zika virus infection and fetal disease.
Role: PI

(b)(4) Streblow) 12/14/2016 – 12/13/2017 (b)(4)
(b)(4) $46,934
Biological Profiling of Anti-CHIKV Recombinant mAbs
The major goal of this project is to determine the efficacy of mAb's directed against CHIKV using in vitro and mouse models.
Role: PI

(NEW)
DARPA-BAA-16-33 (Streblow) 8/29/2017 – 8/28/2018 (b)(4)
(b)(4) /DARPA $470,935
Anti-CHIKV mAb Therapeutics
The goal of the project is to characterize a novel immunotherapeutic directed against CHIKV in NHP.
Role: PI

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

INACTIVE
(b)(4) (Picker) 9/22/2014 – 9/30/2018 (b)(4)
(no-cost extension)
MHC II- and MHC E-restricted CD8+ T Cells and Control of HIV
The goal of this project is to provide fundamental research on a new type of vaccine-elicited CD8+ T cell immunity with the potential to control and clear HIV, and therefore to enable development of a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Project Manager; Outcome 8 Activity 4.3

OTHER SUPPORT: Mark Suto, Ph.D.

ONGOING SUPPORT

Suto (PI) 04/1/2016 – 3/31/2020 No Task Orders
(b)(4) (NCI) BAO# 16XS124
Chemical Biology Consortium — Collaborative Drug Discovery Partnership with NCI. As a specialized center SR will contribute to all aspects of the programs identified by the NCI including screening, medicinal chemistry in vivo studies through final drug development. Role: PI

Whitley (PI) 03/01/2014 – 02/28/2019 (b)(4)
NIH, NIAID (UAB) 1U19 AI109680 $1,025,151
Antiviral Drug Discovery and Development Center. The goals of this NIAID program are the development of antiviral drugs for the treatment of emerging and reemerging infections. Specifically, the focus will be on flaviviruses, alphaviruses, corona viruses and influenza. The goal is to identify compounds working through mechanisms that affect viral replication and develop these leads in a translational manner to new human therapeutics. Role: Co-I, Medicinal Chemistry Core

Murphy-Ullrich (PI) 08/1/2014 – 07/31/2019 (b)(4)
NIH (UAB) 1R01 CA175012-01A1 $1,646,665
Inhibitors of Transforming Growth Factor (TGF)-β for Multiple Myeloma and Fibrotic Conditions. This proposal will combine mechanistic studies with drug discovery efforts to achieve our goal of identifying an orally active lead compound for treatment of Multiple Myeloma. We will further determine the role of the TSP1-TGF-β pathway in MM through use of immune competent and TSP1 null models, by comparison of lead compounds to global TGF-β inhibitors. Role: Co-I

Suto (PI) 09/14/2015 – 09/13/2020 (b)(4)
(b)(4) $1,187,439
Identification of Therapeutics for Cystic Fibrosis. The goal is to identify novel read-through drugs for the treatment of cystic fibrosis. Initially, a large high-throughput screen will be run and the compounds further evaluated in several mechanistic assays. Lead optimization and profiling of the compounds will be initiated to identify a preclinical candidate. Role: PI

Kimberly (PI) 09/01/2015 – 08/31/2020 (b)(4)
NIH, NCATS (UAB) U54 TR001368-01 $6,324,075 (UL1, KL2, TL1)
UAB Center for Clinical and Translational Science (CCTS). The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS support this overall mission in a highly integrative network of relationships. Success in creating such an environment is dependent upon success in achieving five strategic priorities: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability. Role: Co-I

Agarwal (PI) 04/01/2016 – 3/31/2018 (b)(4)
NIH, NIDDK (UAB) R01 DK059600-12 $135,594
Hemeoxygenase-1 (HO-1) and Kidney Disease. The goal is to identify compounds that up-regulate heme-oxygenase 1 and evaluate them in a model of kidney disease. The compounds were identified using a high-throughput screening strategy. Role: Co-I

COMPLETED SUPPORT

Suto (PI) 05/01/2015 – 04/30/2017
(b)(4)

Development of Small Molecules Active at Disease Onset in ALS. The goal is to develop a primary and backup series of small molecules that could be further developed for use in the treatment of ALS. We will identify a primary lead series that is novel, orally bioavailable, and has CNS penetration using a directed medicinal chemistry effort focused on a current lead compound. The best candidates will be evaluated in vivo in the SOD-1 G93A ALS animal model to assess efficacy and target engagement. Role: PI

Suto (PI) 04/27/2009 - 6/26/2015
(b)(4) (NCI) BAO# 28XS124
Chemical Biology Consortium — Collaborative Drug Discovery Partnership with NCI. The goals of this NCI program were to provide a comprehensive research framework leading to the development of new anticancer drugs, focusing on the identification of new molecular targets, and the rapid development of these from medicinal chemistry through final drug development. Role: PI

Suto (PI) 07/14/2014- 7/14/2015
(b)(4)
Targeting Hallmarks of Cancer for Oncology Drug Discovery. The goals of this state-funded initiative were to develop and train newer generations of cancer researchers as well as to promote innovation and research within the state. Role: PI

Matalon (PI) 09/22/14 – 09/22/2016
NIH, NIEHS (UAB) 1R21ES024705-01
Central Role of Heme Oxygenase in Reversing Bromine Morbidity and Mortality. The goals of this application were to establish the role of HO-1 in protecting mice from Br2 induced injury and test the efficacy of these compounds. Role: Co-I

PENDING SUPPORT

(b)(4)

OVERLAP – None.

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. OVERALL CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. OVERALL SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded:
G1 Core B (b)(6); (b)(3);7.pdf
G1 Core C Pathak.pdf
G1 Project 1 Nelson.pdf
G1 Project 2 (b)(6); (b)(3);7.pdf
G1 Project 4 Whitley.pdf
G1 Umbrella Whitley.pdf
G1 Core A Whitley.pdf
G1 Project 3 Streblow.pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave. South BIRMINGHAM AL 35294

Oregon Health & Science University	096997515	OR-003	3181 SW Sam Jackson Park Rd. Portland OR 97239
Vanderbilt University	004413456	TN-005	3319 West End Avenue Suite 100 Nashville TN 37203
THe University of North Carolina at Chapel Hill	608195277	NC-004	Administrative Office Bldg.Suiste 2200 104 Airport Rd, CB 1350 Chapel Hill NC 27599
University of Colorado at Denver	041096314	CO-006	1300 E. 17th Place, Room W1126 Anschutz Medical Campus Bldg 500 Denver CO 80045
Portland VA Research Foundation	827052887	OR-01	3710 SW US Veterans Hospital Rd Portland OR 97239
Southern Research Institute	006900526	AL-006	2000 Ninth Avenue South Birmingham AL 35205
Washington University	068552207	MO-001	Campus Box 8051 660 S. Euclid St. Louis MO 63110
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave South BIRMINGHAM AL 352331806
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave. South BIRMINGHAM AL 35294
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Washington University	068552207	MO-001	Campus Box 8051 660 S. Euclid St. Louis MO 63110

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Not Applicable

Core B: Screening Core

Core specific information

Project 1. Flaviviruses

Dengue virus

Aim 1 (accomplished Year 1): A CPE assay employing a dengue viral stock prepared in insect cells and HEK293 host cells was used to screen a total of 304,810 compound samples. Using an activity threshold of inhibition ≥ 26.25% (mean + 3xSD of all data), 2,240 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Forty-five (45) compounds were confirmed and validated as hits with IC_{50} < 20 μM and no cytotoxicity. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): Compounds are tested at ten concentrations in an immunofluorescence assay measuring viral protein expression in HEK293 host cell. IC_{50} values are calculated from this data as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

West Nile Virus

Aim 1 (accomplished Year 3): A CPE assay was constructed to identify inhibitors of the viral 2'-O-Methyltransferase. The 2'-O-MTase activity of flaviviruses promotes viral evasion of the Ifit family of genes, a group of host cell IFN-stimulated immune effector proteins. In order to detect inhibitors of virus 2'-O-MTase activity, the HTS assay was performed using transformed HEK 293 cells that expressed Ifit1 when induced by doxycycline. Such compounds will promote the host cell defense mechanism and reduce CPE. The assay also detected compounds that had a direct anti-viral effect since those compounds reduced CPE independently of Ifit expression. A total of 197,077 compounds were screened using HEK cells treated with doxycycline to induce ifit1 expression. Using a statistical threshold of inhibition ≥ 19.03% (mean + 3xSD of all data), 2997 compounds were identified as active. In order to confirm hits and distinguish potential inhibitors of 2'-O-MTase activity from those with direct anti-viral activity, the compounds were retested at 10 concentrations for inhibition of CPE and direct cytotoxicity effects in HEK cells treated with or without doxycycline (i.e. with or without ifit1 expression). IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Hits were deemed confirmed and valid if they had an IC_{50} < 75 μM and no cytotoxic effect. By this criteria, 30 compounds were active only if ifit1 was expressed (i.e. active only in cells treated with doxycycline) and were identified as potential inhibitors of the viral 2'-O-Methyltransferase. An additional 130 compounds were active independent of Ifit expression and identified as those having direct anti-viral effects. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): Compounds are tested at ten concentrations in an immunofluorescence assay measuring viral protein expression in TRex293 host cells. IC_{50} values are calculated from this data as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Zika Virus (supplemental)

Aim 1 (accomplished year 4): A CPE assay employing a Zika viral stock prepared in insect cells and Vero CCL81 host cells was used to screen a total of 310,438 unique compounds at a single concentration. From this data, 3200 compounds with inhibition ≥ 51.29 % were selected for retesting at 10 concentrations for both anti-viral CPE and direct cytotoxicity effects for which IC_{50} and CC_{50} values were calculated, respectively. A total of 1090 compounds from the SR collection and 107 compounds from the Gilead collection were confirmed and validated showing concentration dependent inhibition of CPE with no direct cytotoxicity. Clustering analysis was performed on 1028 SR hits after eliminating 59 PAINS and 3 duplicate compounds. This resulted in 660 clusters/singletons as follows:

- 12 clusters with 6 or more than 6 members
- 6 clusters with 5 members
- 12 clusters with 4 members
- 50 clusters with 3 members
- 141 clusters with 2 members
- 439 singletons

Supplemental funding was not awarded for follow up chemistry efforts.

Aim 2 (ongoing): In order to determine compound specificity for different flaviviruses, compounds synthesized for the Dengue and West Nile projects are also tested in an immunofluorescence assay measuring Zika viral protein expression in Vero CCL81 host cells. IC_{50} values are calculated from this data as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Project 2. SARS Corona Virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells selected for expression of the SARS CoV receptor (ACE2; angiotensin-converting enzyme 2) were used to screen a total of 305,648 compound samples. Using an activity threshold of inhibition ≥ 80%, 2,492 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Of these, 307 compounds were confirmed and validated as hits showing IC_{50} < 20 μM and SI (IC_{50}/CC_{50}) > 3. An additional 268 compounds were confirmed and validated as hits showing IC_{50} > 20 μM and SI (IC_{50}/CC_{50}) > 3. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer was developed using a recombinant SARS Nanoluc virus produced in the Baric lab. The assay is employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

Project 3. Alpha Viruses

Chickungunya virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 50.38% (mean + 3xSD of all data), 2,558 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-four (44) hits were confirmed and validated with IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer and replication was developed using a recombinant CHIKV Nanoluc virus produced in the Heise lab. IC_{50} values are calculated from the luminescent signal obtained in virus infected THF cells treated at 10 concentrations of test compound as an index of potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. The nanlouc reporter signal in these wells is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Venezuelan Equine Encephalitis virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 12.12% (mean + 3xSD of all data), 940 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-two (42) hits were confirmed and validated with IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): Compounds are tested at ten concentrations in the CPE assay and IC_{50} values are calculated as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Project 4. Influenza A viruses

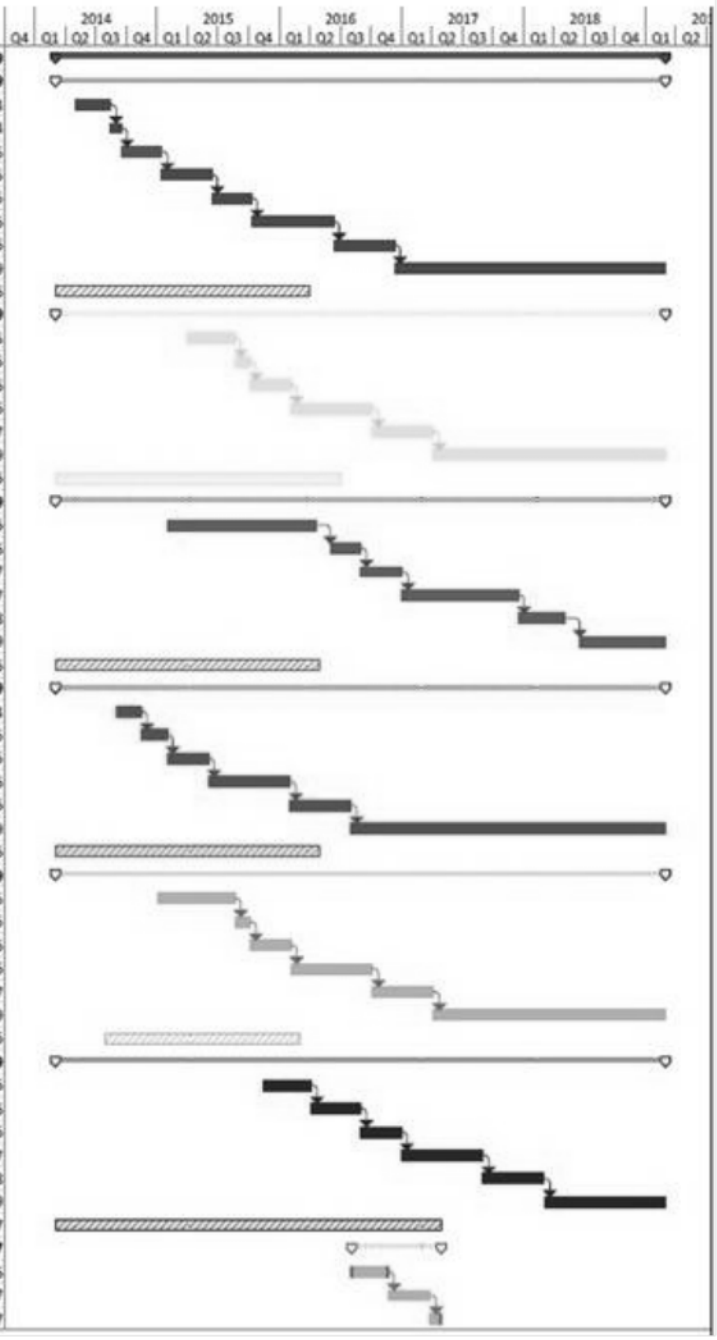
Aim 1 (accomplished years 3-4): An enzyme linked virus inhibitor reporter assay was used for HTS. This assay (described in Lutz et al., J. Virol. Methods 2015, 126: 13-20) utilizes an HEK293 cell line engineered to express virus-like negative sense RNA transcripts encoding firefly luciferase flanked by the untranslated regions of influenza A/WSN/33 NP segment. (ELVIRA® Flu A-luc cells). When these cells are infected by influenza A, the virus RdRp transcribes this RNA into mRNA and luciferase protein is produced. Luciferase enzyme activity is then measured as a reporter of virus infection enabling the anti-viral activity of test compounds to be determined by a decrease in luciferase activity. A total of 196,721 unique compounds were screened in HTS using the H3N2 (Udorn) strain. Using an activity threshold of inhibition ≥ 83.45% (mean + 3xSD of all data), 3200 samples were identified as active and retested at 10 concentrations for anti-viral and direct cytotoxicity effects. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. After eliminating compounds using PAINS filtering, a total of 892 compounds from the SR collection and 185 from the Gilead collection were confirmed and validated showing concentration dependent inhibition of the luciferase reporter signal with no cytotoxicity in ELVIRA® HEK reporter cells. However, when counter screened for CPE in MDCK cells, only 19 compounds showed activity. These results suggest either a high false positive hit rate in the ELVIRA reporter assay or a high false negative reconfirmation rate in the CPE assay. To resolve this issue the 892 hit compounds in the SR collection were retested using an immunofluorescent assay (IFA) measuring M2 protein levels in H3N2 infected A549 cells. Testing each compound at a single concentration in two separate assay runs, 280 compounds were been identified with reproducible activity. These compounds were retested in the IFA assay at 10 concentrations to confirm activity and determine IC_{50} values and counter screened for cytotoxicity effects in the A549 cells. For compounds showing no cytotoxicity on A549 cells as measured by CC_{50} > 40 μM the IC_{50} values for the IFA anti-viral effect are as follows: 12 compounds with IC_{50} < 1 μM, 48 compounds with 1 μM < IC_{50} < 10 μM, and 98 compounds with 10 μM < IC_{50} < 50 μM. The structures of these compounds have been sent to the MCLDC for further evaluation. To identify additional hits, 20,530 proprietary compounds in the SR collection were screened in the MDCK CPE assay. Of these 2,155 compounds were tested in concentration response assays from which six compounds previously known to have anti-viral effects for influenza were identified. An additional 223 compounds were identified from the remaining compounds tested at a single concentration. These compounds were retested for concentration response to confirm activity in the anti-viral CPE assay and counterscreened for cytotoxicity against MDCK cells. Of these, thirty-three (33) were confirmed with IC_{50} < 50 μM and corresponding CC_{50} > 40 μM. These compounds are currently being counter screened in the ELVIRA reporter assay and the IFA assay to further validate the anti-viral effect.

Aim 2 (ongoing): To drive SAR, the immunofluorescent assay measuring surface expression of M2 in H3N2 infected A549 host cells will be used to determine IC_{50} values as an index of compound potency. As with the other projects, media taken from wells treated with 0, 1 and 10 μM test compound will be serially diluted over 8 logs and used to infect fresh ELVIRA cells. The reporter assay will be used to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample will be calculated. The maximal fold

reduction in the log of the number of infectious virus particles will used as an index of compound efficacy.

Timeline of activities

ID	Task Name	Start	Finish
1	**CETR U19**	**Mon 3/3/14**	**Thu 2/28/19**
2	**DEN Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
3	DEN Assay Development	Thu 5/1/14	Thu 8/14/14
4	DEN HTS	Fri 8/15/14	Mon 9/15/14
5	DEN Assay Troubleshooting	Tue 9/16/14	Wed 1/14/15
6	DEN Resume HTS	Thu 1/15/15	Fri 6/12/15
7	DEN Hit Triage	Mon 6/15/15	Fri 10/9/15
8	DEN Lead Series Identification	Mon 10/12/15	Fri 6/10/16
9	DEN Proof of Concept	Mon 6/13/16	Fri 12/9/16
10	DEN Lead Optimization	Mon 12/12/16	Thu 2/28/19
11	DEN Existing Hit Follow Up	Mon 3/3/14	Thu 3/31/16
12	**VEEV Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
13	VEEV Assay Development	Wed 4/1/15	Fri 8/21/15
14	VEEV HTS	Mon 8/24/15	Mon 10/5/15
15	VEEV Hit Triage	Tue 10/6/15	Thu 2/4/16
16	VEEV Lead Series Identification	Fri 2/5/16	Wed 10/5/16
17	VEEV Proof of Concept	Thu 10/6/16	Thu 4/6/17
18	VEEV Lead Optimization	Fri 4/7/17	Thu 2/28/19
19	VEEV Existing Hit Follow Up	Mon 3/3/14	Thu 6/30/16
20	**H3N2 Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
21	H3N2 Assay Development	Mon 2/2/15	Wed 4/20/16
22	H3N2 HTS	Thu 6/2/16	Wed 8/31/16
23	H3N2 Hit Triage	Thu 9/1/16	Mon 1/2/17
24	H3N2 Lead Series Identification	Tue 1/3/17	Fri 12/15/17
25	H3N2 Proof of Concept	Mon 12/18/17	Fri 5/4/18
26	H3N2 Lead Optimization	Mon 6/18/18	Thu 2/28/19
27	H3N2 Existing Hit Follow Up	Mon 3/3/14	Fri 4/29/16
28	**SARS CPE (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
29	SARS CPE (BSL3) Assay Development	Mon 9/1/14	Fri 11/14/14
30	SARS CPE (BSL3) HTS	Mon 11/17/14	Fri 1/30/15
31	SARS CPE (BSL3) Hit Triage	Mon 2/2/15	Wed 6/3/15
32	SARS CPE (BSL3) Lead Series Identification	Thu 6/4/15	Tue 2/2/16
33	SARS CPE (BSL3) Proof of Concept	Wed 2/3/16	Mon 8/1/16
34	SARS CPE (BSL3) Lead Optimization	Tue 8/2/16	Thu 2/28/19
35	SARS CPE (BSL3) Existing Hit Follow Up	Mon 3/3/14	Fri 4/29/16
36	**CHIKV (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
37	CHIKV (BSL3) Assay Development	Mon 1/5/15	Fri 8/21/15
38	CHIKV (BSL3) HTS	Mon 8/24/15	Mon 10/5/15
39	CHIKV (BSL3) Hit Triage	Tue 10/6/15	Thu 2/4/16
40	CHIKV (BSL3) Lead Series Identification	Fri 2/5/16	Wed 10/5/16
41	CHIKV (BSL3) Proof of Concept	Thu 10/6/16	Thu 4/6/17
42	CHIKV (BSL3) Lead Optimization	Fri 4/7/17	Thu 2/28/19
43	CHIKV (BSL3) Existing Hit Follow Up	Fri 8/1/14	Mon 2/29/16
44	**WNV (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
45	WNV (BSL3) Assay Development	Mon 11/16/15	Tue 4/5/16
46	WNV (BSL3) HTS	Wed 4/6/16	Wed 8/31/16
47	WNV (BSL3) Hit Triage	Thu 9/1/16	Fri 12/30/16
48	WNV (BSL3) Lead Series Identification	Mon 1/2/17	Thu 8/31/17
49	WNV (BSL3) Proof of Concept	Fri 9/1/17	Fri 3/2/18
50	WNV (BSL3) Lead Optimization	Mon 3/5/18	Thu 2/28/19
51	WNV (BSL3) Existing Hit Follow Up	Mon 3/3/14	Fri 4/28/17
52	**ZIKV (Supplimental Funding)**	**Fri 8/5/16**	**Fri 4/28/17**
53	ZIKV Assay Development	Fri 8/5/16	Mon 11/21/16
54	ZIKV HTS	Tue 11/22/16	Mon 3/27/17
55	ZIKV Hit Triage	Tue 3/28/17	Fri 4/28/17



Core C: Medicinal Chemistry and Lead Development Core (MCLDC)

Core Specific Information.

A detailed summary of Core C interactions with all four individual Research Projects (1-4) and Core B with respect to the activities as described in original grant proposal are as below.

1. **Research Project 1 (Flaviviruses):**

DENV: Continuing from Year 3 efforts on three re-confirmed hits from AD3C HTS screen a total of ~125 analogs were synthesized and tested for antiviral potency (EC_{90}) and efficacy (VTR) in MB assay at SR-HTS and Research Project-1 site using immunofluorescent based DENV-2 VTR assay. The three hits for lead optimization chemistry were SRI-35847 [EC_{90} = 2.9 μM, CC_{50} >30μM, VTR = 3.5 logs]; SRI-33361 [EC_{90} = 0.9 μM, CC_{50} >5μM, VTR = 3.3 logs] and SRI-33376 [EC_{90} = 1.6 μM, CC_{50} = 3.5 μM, VTR = 3.6 logs]. ADME properties on these compounds were: SRI-35847 [mouse liver microsomal stability (MLM) t½ = 4 min and solubility = 13 μM]; SRI-33361 [MLM t½ = 1 min and solubility = 9 μM] and SRI-33376 [MLM t½ = 1.6 min and solubility = 15.5 μM]. A small set of analogs of SRI-35847 were synthesized to determine if this hit warranted further structural development for improved antiviral potency and efficacy. A total of 16 specific substituted analogs of the primary core of the hit compound were synthesized to generate SAR and are currently being evaluated for antiviral activity by Research Project-1 as well as in MB assay. Upon collecting data on antiviral potency (EC_{90}) and efficacy (VTR) of these analogs, a medicinal chemistry plan will be evaluated.

SRI-33361 showed excellent antiviral potency and efficacy [EC_{90} = 0.9 μM and VTR = 3.3 logs], but possessed cytotoxicity (CC_{50} >5μM).However, chemists have identified some functional groups in the molecule that may be responsible for cytotoxicity and have designed and synthesized 38 analogs which were submitted to Research Project-1 for potency (EC_{90}) and VTR assays as well as to Assay Development Core (Core B) for MB assay. After evaluating the viral activity data on the analogs, it was concluded that the antiviral activity was very closely related to cytotoxicity. Non-toxic analogs showed complete loss of antiviral activity. Also, any reasonable changes to the structure of hit SRI-33361 to improve ADME properties [aqueous solubility, log D, and mouse and human microsomal stability] resulted in loss of antiviral activity. Hence, further work on this series was abandoned.

Lead optimization efforts was carried out on SRI-33376 [EC_{90} = 1.6 μM, CC_{50} = 3.5 μM, VTR = 3.6 logs]. The major issue with this lead compound was cytotoxicity towards HEK293 cells associated with modest antiviral potency and very low microsomal stability [MLM t½ = 1.6 min and solubility = 15.5 μM]. Specifically, a tryptophan moiety was considered as a major source of cytotoxicity. However, replacement of this group did not yield antiviral potency. Only moderate activity (EC_{50} = 5–15 μM) was observed in some of the analogs devoid of tryptophan. After synthesizing 50 analogs, further work on this series was abandoned.

WNV: After the completion of HTS screening campaign on 197K+ compounds at the end of the 4th quarter of Year 3, 21 hits were purchased from commercial sources in Year 4 and analyzed by Core C before submitting to Research Project-1 site for re-confirmation activity in immunofluorescent based VTR assay. The HTS screens were performed in the following two ways: ***Screen-A***) Targeted mechanism: Inhibition 2'-*O*-Methyltransferase A, and ***Screen-B***) Secondary mechanism: Direct antiviral effect. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability were also evaluated on all reconfirmed hits with VTR >2 logs. Three hits from each assay were reconfirmed and medicinal chemistry on 2 hits from direct CPE assays were carried out by Core C: SRI-37776 [EC_{90} = 0.9 μM, CC_{50} = 12 μM, VTR = 2.9 logs] and SRI-37710 [EC_{90} = 3.8 μM, CC_{50} >30 μM, VTR = 2.3 logs]. ADME properties on these compounds were: SRI-37776 [MLM t½ = 8.7 min and solubility = 9.7 μM] and SRI-37710 [mouse microsomal stability: t½ = 5.6 min and solubility = 10.9 μM]. A set of analogs of SRI-37776 were synthesized to determine if this hit warranted further structural development for improved antiviral potency, efficacy and ADME properties. A total of 61 diverse substituted analogs of the primary core were synthesized and evaluated for antiviral activity by Research Project-1 as well as in the MB assay at SR-HTS. Most of these compounds showed no improvement in efficacy (VTR in logs) as compared to initial hit SRI-37776 and some compounds completely lost antiviral activity. Microsomal stability in a few analogs increased, but solubility remained a great concern. This series was abandoned and the chemist was moved to work on hit SRI-37710. A total of 18 specific compounds have been synthesized so far around the core structure of SRI-37710 and data is currently being analyzed.. Lead optimization will be done based on the current data set.

ZIKA: HTS screen on Zika virus was also done in Years 3-4 under supplemental funding to the current funding. Dose response data was analyzed by Core C. A total of 1090 hits were analyzed in PAINS filtration followed by clustering analysis. From the active set, 59 compounds were filtered through PAINS filtration and 3 compounds were duplicate. Clustering analysis was done on the remaining 1028 hits from which 439 singletons and 221 clusters were identified. From these 221 clusters, 18 clusters had > 5 members. The data on these compounds were sorted by EC_{50} and Selectivity Index (SI = CC_{50}/EC_{50}), and compounds with EC_{50} < 10 μM and SI > 20 were selected as potential hits. Some compounds were also filtered by visual inspection of structures possessing unwanted functional groups, by core structure uniqueness and for commercial availability. A total of 27 compounds were selected for re-confirmation in the ZIKA antiviral assay. The funding only supported the screening and hit triage; thus, no further testing against ZIKA virus was carried out. However, since the ZIKA virus belongs to the same virus family as WNV and DENV, we repurchased fresh samples of these 27 hits to test against the WNV and DENV viruses. The fresh samples that were commercially obtained were tested for their purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting to the WNV and DENV assays at Research Project-3 site and MB assay at SR-HTS group. Some of the compounds showed only moderate potency (EC_{90}) and efficacy (VTR) activity. None of the compounds were carried forward in hit to lead chemistry for WNV and DENV viruses.

2. Research Project 2 (Coronaviruses)

Chemistry efforts continued in Year 3 on SRI-35293 [ADR EC_{90} = 2.5 μM, NL EC_{50} = 6.8 μM, CC_{50} > 30 μM, VTR > 3 logs] HTS hit. A new analog SRI-36565 with an EC_{90} = 0.8 μM and CC_{50} > 30 μM in the NanoLuc (NL) assay against SARS virus was achieved. This compound was submitted to Research Project-2 site for its potency in a CPE assay and efficacy in a VTR assay using VeroE6 cells. Meanwhile, based on the NL assay results on SRI-36565, a hit-to-lead campaign was initiated to improve potency, efficacy and ADME properties with target values, EC_{50} <500 nM, EC_{90} <1 μM), CC_{50} > 20 μM, MLM $t_{1/2}$ > 60 min and solubility ≥10 μM. Around 30 analogs were synthesized and evaluated for antiviral activity in SAR-NL assay. Most of these compounds showed no improvement in potency (EC_{50}) as compared to initial hit SRI-36565 and some compounds completely lost antiviral activity. SRI-36565 only showed ~1 log reduction in viral titers at 10 μM concentration from Research Project-2 site. Inconsistent results were obtained from NL assay and VTR assay which put a hold on further development of this series.. It took a while to standardize the assay conditions at Research Project-2 sites. Once the VTR assay was available, five compounds were selected for testing in the VTR assay using VeroE6 cells and human airway epithelial (HAE) cultures for hit a optimization campaign to improve ADME properties, potency and efficacy in the ranges described above. These five compounds were SRI-35020 [EC_{50} = 5-6 μM, CC_{50} >30 μM]; SRI-33684 [EC_{50} = 0.5-1.8 μM, CC_{50} >30 μM]; SRI-36096 [EC_{50} = 4.7-6.7 μM, CC_{50} >30 μM]; SRI-36097 [EC_{50} = 3.5-5.8 μM, CC_{50} >30 μM] and SRI-36100 [EC_{50} = 4.4-5.3 μM, CC_{50} > 30 μM]. ADME properties on four of these compounds were: SRI-35020 [MLM t½ = 1.7 min]; SRI-33684 [MLM t½ = 22 min and solubility <1 μM]; SRI-36096 [MLM t½ = 30 min and solubility = 100 μM] and SRI-36097 [MLM t½ = 7.9 min and solubility = 90.8 μM]. Recently, virus plaque reduction data @ 10 μM from Research Project–2 sites using VeroE6 cells and human airway epithelial (HAE) cultures were obtained. SRI-35020, SRI-33684 and SRI-36096 showed <1 log reduction in viral plaques. However, SRI-36097 and SRI-36100 showed 3.5 and 2.5 logs reduction of viral plaques, respectively. Currently, dose dependent virus plaque reduction assay is being carried out at Research Project–2 site. This data provided us more confidence in pursuing a hit to lead campaign, and thus 41 closely related analogs of SRI-36097 will be ordered for screening in the NL assay. Compounds possessing EC_{50} < 10 μM will supplied to Research Project-2 site for its potency in a CPE assay and efficacy in a VTR assay using VeroE6 cells. Compounds with virus plaque reduction >2 logs will be tested in virus plaque reduction assay using HAE cultures. When these results are received, a lead optimization campaign will start that will target compounds with improved ADME properties, potency and efficacy in the ranges described above.

3. Research Project 3 (Alphaviruses)

VEEV: Core C continued chemistry from Year 3 on a MLPCN re-confirmed hit, SRI-33394, which showed excellent antiviral activity in a Normal Human Dermal Fibroblasts (NHDF) cell line against VEEV (EC_{90} = 0.7 μM, CC_{50} > 30 μM and VTR = 6.5 logs). However, this compound had very low mouse microsome stability (t½ = 2.1 min). This instability may likely be attributed to the presence of a thiourea, furan functionality and

ethyldimethylamine side chain in the molecule. From several analogs synthesized in Year 3, one compound from this series, SRI-36129 (replacing the thiourea with thiazole in SRI-33394) exhibited decent antiviral potency [EC_{90} = 1.33 μM], efficacy [VTR = 9.5 logs] and reasonable ADME properties [MLM t½ = 21.6 min and solubility = 51.5 μM]. It provided a path forward to replace the microsomal unstable thiourea to a thiazole. In Year 4, 15 analogs were synthesized in which the ethyldimethylamine side chain in SRI-33394 was modified while keeping the thiourea constant. From this exercise, SRI-36959 was identified that retained the antiviral potency and efficacy [EC_{90} = 0.78 μM, CC_{50} > 30 μM and VTR = 5.5 logs]. As expected SRI-36959 had poor mouse microsomal stability [MLM t½ = 1.6 min] because of thiourea functionality. The medicinal chemistry approach has now turned to identifying replacements of the thiourea functionality as well as replacing the ethyldimethylamine side chain with pyrrolidines and replacing the furan with substituted phenyl rings. It is anticipated that these new analogs in Year 5 to improve drug like properties such as mouse microsomal stability [MLM t½ >30 min], excellent antiviral activity [EC_{90} < 1 μM] and efficacy [VTR > 5 logs] *in vitro* to generate lead molecule for mouse animal model studies.

Hit to lead and lead optimization continued from Year 3 on two reconfirmed hits, SRI-36427 and SRI-37938, identified from VEEV HTS campaign.. SRI-36427 was selected for optimization based on its antiviral and VTR activity (EC_{90} = 0.25 μM; CC_{50} > 50 μM; VTR = 10 logs at 12.5 μM with no virus at 25 μM). SRI-36427 was very unstable in mouse liver microsomes (t½ < 2 min) but had reasonable solubility (25 μM). Medicinal chemistry continued with a goal to identify a compound with improved mouse microsomal stability (t½ > 30 min) and solubility (> 10 μM) and to identify a compound to be tested in animals for antiviral efficacy and potency. Fifty-four diverse analogs of SRI-36427 were synthesized that focused on eliminating potential metabolic hot spots in the molecule while retaining potency and efficacy. These compounds were tested at Research Project-3 site in a CPE-based VTR assay as well as at SR-HTS in CPE-VTR combo assay. All analogs exhibited a range of potency and efficacy but none of the modifications increased metabolic stability Thus, lead generation efforts of hit SRI-36427 were abandoned and a research article will be published with results.

CHIKV: Core C also continued with medicinal chemistry efforts from Year 3 of the project to develop a lead compound from a previous MLPCN VEEV screen hit, SRI-33366, which showed EC_{90} = 3.2 μM; CC_{50} > 50 μM and VTR = 1.7 logs against CHIKV. A total of 55 new analogs were synthesized and tested for antiviral activity and potency. Two compounds (SRI-34963 and SRI-36498) were also synthesized in large scale (>2 g) for *in vivo* and animal experiments. The new reverse amide analog SRI-36498 [EC_{90} = 0.77 μM, CC_{50} > 18 μM and VTR = 3.2 logs] became the new lead molecule with improved MLM [t½ =57 min] but still possesses very low solubility (<1 μM) in PBS at pH 7.4. Several formulations were tried to carry out *in vivo* pharmacokinetic (PK) studies via oral (5 and 10 mg/kg), subcutaneous (5 mg/kg), intraperitoneal (5 mg/kg) and intravenous (1 mg/kg) administrations in male C57BL/6 mice. Finally, 10%NMP/3% Solutol in 30%HP-β-CD was used to carry out *in vivo* PK studies. Analysis of the PK data showed that the subcutaneous route of administration [t½ = 6.6 h, AUC = 1253 h*ng/mL, C_{max} = 681 ng/mL] was preferred and thus, a mouse efficacy study was performed at Research Project-3 site [BID dose of 20 mg/Kg for three days]. Both male and female C57BL/6 mice were used.. Mixed results were obtained in which the female mice did not show any efficacy. However, male mice showed medium efficacy. Analysis of various tissue samples (spleen, L & R muscles, L & R ankles) showed compound distribution in tissues in low concentrations. Protein binding study showed >99% bound. We were not able to dissolve SRI-36498 in > 20 mg/kg dose using the same formulation for animal testing at higher dose. Several analogs of SRI-36498 were designed and synthesized to increase solubility and are being tested *in vitro* for their antiviral potency and efficacy at Research Project-3 site. Based on the *in vitro* antiviral activity results and *in vivo* PK data, the best compound will be tested in mice @ 80 mg/Kg dose for efficacy.

After the completion of the HTS screening campaign for CHIKV on 197K+ compounds (Year 3), dose response data was analyzed by Core C. A total of 11 hits from the HTS screen that were reconfirmed using the CPE-based antiviral assay were then tested in the VTR assay. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all confirmed hits. These results helped us to prioritize these compounds before initiating medicinal chemistry. Lead generation chemistry started on SRI-33001 [EC_{90} = 0.93 μM; CC_{50} > 40 μM; VTR = 6.9 logs at 6.25 μM]. ADME properties were also evaluated on SRI-33001 [MLM t½ = 27.1 min; solubility < 1 μM]. Design and synthesis of analogs of SRI-33001 was carried out by Core C and to date, a total of 47 compounds were designed and synthesized. Initial hit SRI-33001 are being optimized for potency (EC_{90} < 1 μM), efficacy (>3.5 logs) and ADME properties [MLM t½ > 30 min; solubility > 10 μM] to have a compound for animal efficacy studies. However, medicinal chemistry is also

being planned for analogs of SRI-36767 [EC_{90} = 0.1 μM; CC_{50} > 40 μM; VTR = 6.9 logs at 6.25 μM; MLM t½ = 2 min; solubility = 58 μM], as a back-up series, with the goal of identifying a lead compound that has improved mouse microsomal stability (t½ > 30 min) and solubility (>10 μM) while keeping the potency and efficacy in desired range [EC_{90} < 1 μM; VTR > 3.5 logs]. The Synthesis of analogs of SRI-36767 will soon start and carry into Year 5 Core C efforts.

In an attempt to identify some cross virus active molecules within the same virus family, the re-confirmed hits from VEEV and CHIKV hits were tested in the different virus assays. From this testing, two compounds were identified that showed potential activity versus both viruses. These two compounds, SRI-36426 and SRI-36768, are considered dual inhibitors and were further tested in both the CPE and VTR assays against both viruses. The results for SRI-36426 were: VEEV [EC_{90} = 0.72 μM; CC_{50} > 50 μM; VTR = 6 logs at 12.5 μM and no virus at 25 μM]; CHIKV [EC_{90} = 1.2 μM; CC_{50} > 50 μM; VTR = 3 logs at 12.5 μM] and the results for SRI-36768 were: VEEV [EC_{90} = 0.7 μM; CC_{50} > 30 μM; VTR = 6 logs at 10 μM]; CHIKV [EC_{90} = 0.2 μM; CC_{50} > 30 μM; VTR = 7.6 logs at 0.78 μM]. The ADME properties for each of these compounds were also determined: [SRI-36426: mouse microsomal stability: t½ = 5 min; solubility = 26 μM and SRI-36768: mouse microsomal stability: t½ = 3 min; solubility = 2 μM]. Lead generation studies were continued on SRI-36426 from Year 3 by Core C chemists. A total of 62 diverse analogs have been designed and synthesized so far to generate preliminary SAR. These analogs were submitted to Core B to screen in a combination assay of antiviral effect (EC_{50} and VTR) as well as for testing at the Research Project-3 site in a VTR assay. So far, few analogs have shown an improvement in microsomal stability and solubility while maintaining acceptable potency and efficacy. Hit to lead development is in a very early stage for this chemical series and will continue in Year 5 of this project. However, structural changes to SRI-36768 with the goal to improve the compound's ADME properties will be synthesized in Year 5 of the project. Based on the activity and ADME profiles from each series, one lead compound will be further optimized with the goal of identifying a compound to test in animals.

The structural biology group also continued work on target identification and binding studies on SRI-34963 from Year 3 which were based on preliminary data that has been generated from virus resistant studies done at Research Project-3 sites. Biolayer interferometry (BLI) technique was used to detect the binding of macrodomain of nsP3, nsP3(1-160) to SRI-34963. SRI-34963 showed non-specific binding to different sensors including Ni-NTA sensors and anti-penta His sensors, which caused difficulty in determining the binding affinity of SRI-34963 to nsP3(1-160). SRI-34963 was co-crystallized with nsP3(1-160) or soaked into the nsP3(1-160) crystals. The structures of nsP3(1-160) were determined at about 1.5Å. No extra density was found for SRI-34963 in the nsP(1-160) structures. The failure to observe the compound structure could be due to the poor solubility of SRI-34963. nsP3 (1-160) protein will be used to set up mono-ADP-ribosylhydrolase assays which can be used for high-throughput screening. High quality nsP3(1-160) crystals will be also used for fragment-based screening to further develop compounds that inhibit mono-ADP-ribosylhydrolase activity in the nsP3 macrodomain.

4. Research Project 4 (Influenza A virus)

A fresh solid sample of each of the 19 hits, identified in the third quarter of Year 3 from the HTS screen against H1N1 and H3N2 strains using MDCK cells, were evaluated for purity and structural confirmation through HPLC, HR-MS and proton NMR analysis. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability were also evaluated on these 19 re-confirmed hits before prioritizing for hit to lead chemistry.. From the 19 compounds submitted, 7 hits, with EC_{90} in the range of 0.7-19 μM were re-confirmed in a 48 hour VTR assay against H1N1 and H3N2 strains using MDCK cells with no cytotoxicity observed. These 7 hits were further evaluated for their polymerase inhibitory effect in MDCK cells and 3 hits were found to be active: SRI-38369 [EC_{50} = 0.3 μM, CC_{50} = 18 μM], SRI-38409 [EC_{50} = 5 μM, CC_{50} > 30 μM] and SRI-31966 [EC_{50} = 0.8 μM, CC_{50} > 30 μM]. SRI-38369 and SRI-38409, which are structurally related, were selected for follow-up chemistry and a total of 29 analogs were either synthesized or purchased. However, none of these 29 analogs were found to be active when evaluated for their antiviral activity in CPE and VTR assays. Synthesis of analogs of hit SRI-31966, which is a known potent CDK inhibitor, was initially planned, but due to synthetic challenges and instability of the target analogs, this effort was suspended. The 19 hits fidentified from the initial HTS screen from Year 3 were also tested in the 72 hour CPE assay against H3N2

using MDCK cells and none of the compounds showed any significant antiviral activity. Core C did not pursue the 3 re-confirmed hits.

In Year 4, HTS also carried out a screening campaign on a 21K+ SR proprietary compound collection using a CPE assay against H3N2 and MDCK cells. Compounds with >25% inhibition were tested in a CPE antiviral dose response assay and a cytotoxicity assay. Results from these assays gave 3 compounds with EC_{50} <10 μM and CC_{50} >30 μM. HTS also retested 892 hits that were identified from the Year 3 ELVIRA assay in a newly developed Mirror Ball M2 Immunofluroescent assay (IFA) in H3N2 virus using A549 cells. Compounds with >25% inhibition were tested in a dose dependent IFA assay and a cytotoxicity assay. Analysis of these data and results by Core C yielded 20 small molecule hits, with EC_{50} values in the range 0.3-14 μM and CC_{50} >30 μM. , Six nucleoside-based hits with EC_{50} range 0.2-7 μM and CC_{50} > 30 μM were also identified and could be considered for re-confirmation studies. Combining results from above described two studies, a fresh solid sample of these 23 small molecule hits is being acquired which will then be evaluated for purity (HPLC) and integrity (HR-MS and ^{1}HNMR). These samples will be submitted for antiviral activity against H3N2 virus in IFA SAR assay and for cytotoxicity.. Confirmed hits will then be evaluated in a polymerase assay. ADME properties will also be evaluated on all confirmed hits to help in the selection of the two best hits to initiate chemistry on in Year 5.

G1 Project 1 – Nelson

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

The flaviviruses are associated with significant morbidity, mortality, and economic burden throughout world. Nevertheless, no specific anti-viral therapies for disease associated with these viruses are currently available. This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G1 Project 2 – (b)(6); (b)(3):7 U.S.C. § 8401

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

In this Project we will use extensive small molecule libraries and a sensitive high-throughput in vitro screening assay to identify inhibitors of SARS-CoV replication fidelity and RNA capping that will lead to profound in vivo attenuation, and potentially represent broadly-efficacious inhibitors of endemic and emerging CoVs.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G1 Project 4 – Whitley

1. Significant Changes in Specific Aims

Not Applicable.

2. Significance of the Work

Influenza A viruses continue to emerge from the aquatic avian reservoir and cause seasonal epidemics and infrequent pandemics. Recent experimental evidence by our group and others support the development of novel antivirals targeting the influenza polymerase function. The discovery of molecules that inhibit influenza virus RNA replication is essential to complement the existing drug arsenal, which is proving less effective due to the increasing incidence of mutational resistance.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G.1 Umbrella – Whitley

1. Product Development

Not Applicable

2. Biocontainment/Security:

Project 1:

1. Project title: Identification and characterization of anti-flaviviral compounds
2. Project leader: Jay Nelson
3. Collaborators: Alec Hirsch, Jessica Smith (OHSU); Michael Diamond (Washington Univ)
4. BSL laboratory employed: BSL3 (at both OHSU and Washington University)
5. Pathogens evaluated: WNV, DENV, ZIKV

Project 2:

1. Project Title: Inhibitors of coronavirus fidelity and cap methylation as broadly applicable therapeutics
2. Project Leader (b)(6); (b)(3):7 U.S.C. § 8401 MR (Vanderbilt),
3. Collaborator(s): Baric, RS (UNC)
4. BSL Laboratory Employed: BSL3 (Vanderbilt) BSL3/ABSL3 (UNC)
5. Pathogen(s) Evaluated: Both Vanderbilt and UNC: SARS-CoV, MERS-CoV. Both Vanderbilt and UNC Select Agent Certified

Project 3

1. Project title: Novel therapeutic strategies targeting re-emerging alphaviruses
2. Project Leader: Daniel Streblow (OHSU)
3. Project Collaborators: (b)(6); (b)(3):7 U.S.C. § 8401 (UNC), Victor DeFilippis (OHSU) and Thomas Morrison (U Colorado Denver)
4. Labs at the following sites were used:
    a. Vaccine & Gene Therapy Institute/Oregon Health & Science University.
        i. BSL-3/VGTI rm2215A, Small Animal ABSL-3/VGTI, Nonhuman Primate ABSL-3 Building/ONPRC
        ii. Pathogen: Chikungunya Virus
    b. University of North Carolina-Chapel Hill.
        i. (b)(3):7 U.S.C. § 8401
        ii. Pathogen: Chikungunya virus, Venezuelan equine encephalitis virus
    c. University of Colorado-Denver.
        i. BSL-3/UCD Anschutz Medical Campus, Small Animal ABSL-3/UCD Anschutz Medical Campus
        ii. Pathogen: Chikungunya Virus

Core B utilized the BSL3 facility at Southern Research for the following projects:

Project number: 1.2
Project Title: Identification and Development of Anti-Flavivirus Lead Drug Candidates
Project Leaders: Michael Diamond
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogens Evaluated: West Nile (NY99)

Project number: 2

Project Title: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics
Project Leaders: (b)(6); (b)(3):7 U.S.C. § 8401 Ralph Baric
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogens Evaluated: SARS Toronto-2 and SARS Urbani/Nanoluc Clone

Project number: 3
Project Title: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
Project Leaders: Daniel Streblow (b)(6); (b)(3):7 U.S.C. § 8401
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogen Evaluated: CHIKV Sri Lanka strain

3. Follow-on Funding

NIAID R01AI132178. Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
Ralph Baric and Tim Sheahan, Co-P.I. 8/9/17-8/8/22

Goals: Studies with GS-5734 (remdesivir) resulted new R01 via the RFA: Partnerships for Countermeasures Against Select Pathogens [RFA-AI-16-034]. The partnership of UNC, VUMC, Gilead, and UTMB will continue to move remdesivir through preclinical development toward IND submission.

(b)(6); (b)(3):7 U.S.C. § 8401

Goals (b)(6); (b)(3):7 U.S.C. § 8401 (Vanderbilt) has performed significant studies (paper submitted) of remdesivir activity and resistance, and EIDD-1931 activity. (b)(6); (b)(3):7 U.S.C. § 8401 to continue these studies with remdesivir and EIDD-1931. (b)(6); (b)(3):7 U.S.C. § 8401 serves as Scientific Mentor on the program.

G1 Core A Special Requirements

The Admin Core has served all Projects and Cores by the following activities:

- Coordinated preparation and execution of year four annual subaward amendments for all sites.
- Processed payments to sites following submission of invoices.
- Planned and hosted the fourth annual AD3C meeting in which all personnel got to meet each other in person, thereby facilitating further interactions between projects and cores.
- Communicated updates related to NIH grant issues, particularly those that impact the areas being investigated by AD3C investigators
- Provided all necessary materials and cooperation for the internal financial audit of the grant account by the University of Alabama system
- Provided feedback from the External Advisory Board reviews to the Projects and Cores.

G1 Project 3 – Streblow

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

Our Program aims to develop novel antiviral agents to emerging human viral pathogens. This Project will develop broad-spectrum nucleoside/nucleotide inhibitors against Alphaviruses with a focus on Chikungunya virus and Venezuelan Equine Encephalitis virus, both of which are human pathogens that cause severe disease and are associated with mortality and with no currently FDA approved vaccine or therapeutics for treatment.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

1. **THF-ΔIRF-3:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells constitutively express the reverse Tet-transactivator via lentivector (Clontech # 631069). The IRF3 gene sequence has been disrupted using the CRISPR/Cas9 system (AddGene vector # 49535). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 cells per vial. Cryopreserved cells can be brought up directly into a T75 + 14 mL media. Once confluent, cells can be subcultured at 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell line constructed by Dr. DeFilippis.
2. **THF-ΔIFIT1, THF-ΔIFIT2, THF-ΔSTING, THF-ΔMAVS, THF-ΔMAVS/STING, THF-ΔTRIF, THF-ΔIFNAR, THF-ΔSTAT1, THF-ΔMAVS/TRIF, THF-ΔPKR, THF-ΔRIG-I, THF-ΔMDA5, THF-ΔRIG-I/MDA5, THF-ΔIKKβ:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells are also stably transduced with a firefly luciferase-coding region under the control of the interferon responsive element using a lentivector obtained from System Biosciences. Individual cell lines were constructed in which the protein coding regions for IFIT1, IFIT2, TRIF, IFNAR, STING, MAVS, MAVS/STING, MAVS/TRIF, PKR, RIG-I, MDA5, RIG-I/MDA5, IKKβ, or STAT1 were disrupted using the CRISPR/Cas9 system (AddGene vector # 52961). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 cells per vial. Cryopreserved cells can be brought up directly into a T75 + 14 mL media. Once confluent, cells can be subcultured at 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell lines constructed by Dr. DeFilippis.
3. **CHIKV Caribbean Strain Infectious Clone:** $CHIKV_{99659}$ was recently isolated from the British Virgin Islands in December of 2013. A low-passage stock of this strain was provided to the members of the Alphavirus group from Dr. Michael Diamond (Project 2). The Heise lab, in collaboration with Dr. Nathanial Moorman at UNC, has sequenced the isolate and constructed an infectious cDNA clone of the virus. This clone, and derivatives containing viral variants in the Opal termination codon and 3' UTR has been distributed to the other labs within the Program and is generally available to the scientific community upon request.
4. **$CHIKV_{181/25}$ Strains Expressing nano-Luciferase (nLuc):** Into the infectious clone of $CHIKV_{181/25}$ was introduced an in-frame nLuc reporter gene. Two different viruses were constructed by the Heise Lab: pTH1.2 (NSP3-nLuc) and pTH2.1 (Capsid-nLuc), which will be utilized by SR for cherry-pick validation screens and for mechanism of action studies.
5. **$CHIKV_{AF15561}$ strain expressing mKate:** An in-frame mKate reporter gene was cloned into the infectious clone of the pathogenic parental virus of $CHIKV_{181/25}$ ($CHIKV_{AF15561}$). Constructed by Dr. Morrison's group.
6. **G10:** A novel small molecule (4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-

benzo[b][1,4]thiazine-6-carboxamide) capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

7. **AV-C:** A novel small molecule 1-(2-fluorophenyl)- 2-(5-isopropyl-1,3,4-thiadiazol-2-yl)-1,2-dihydrochromeno[2,3-*c*]pyrrole-3,9-dione capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

Composite Application Budget Summary

Categories	Budget Period
Salary, Wages and Fringe Benefits	188,342
Equipment	0
Travel	37,659
Participant/Trainee Support Costs	0
Other Direct Costs (excluding Consortium)	45,613
Consortium Costs	6,713,632
Direct Costs	6,985,246
Indirect Costs	127,658
Total Direct and Indirect Costs	7,112,904

*This application includes at least one component led by an organization that has a DUNS different than the Applicant Organization. The indirect cost calculation for the applicant organization ma not include all allowed Indirect Costs for the first $25K of requested consortium costs and, therefore, may appear less than expected. No action is required from the applicant; NIH will make any appropriate corrections to the budget calculations administratively. The application review will not be affected.

Component Budget Summary

Components	Categories	Budget Period
5064-001 (Admin Core)	Salary, Wages and Fringe Benefits	91,735
	Equipment	0
	Travel	34,659
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	30,708
	Consortium Costs	0
	Direct Costs	157,102
	Indirect Costs	73,837
TOTALS	Total Direct and Indirect Costs	230,939
5069-001 (Core)	Salary, Wages and Fringe Benefits	231,792
	Equipment	0
	Travel	2,563
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	274,579
	Consortium Costs	0
	Direct Costs	508,934
	Indirect Costs	453,275
TOTALS	Total Direct and Indirect Costs	962,209
5070-002 (Core)	Salary, Wages and Fringe Benefits	738,781
	Equipment	0
	Travel	5,125

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	263,382
	Consortium Costs	0
	Direct Costs	1,007,288
	Indirect Costs	1,321,127
TOTALS	Total Direct and Indirect Costs	2,328,415
5071-001 (Project)	Salary, Wages and Fringe Benefits	84,424
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	73,446
	Consortium Costs	0
	Direct Costs	160,870
	Indirect Costs	84,457
TOTALS	Total Direct and Indirect Costs	245,327
5072-002 (Project)	Salary, Wages and Fringe Benefits	157,914
	Equipment	0
	Travel	6,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	140,457
	Consortium Costs	0
	Direct Costs	304,371
	Indirect Costs	158,273
TOTALS	Total Direct and Indirect Costs	462,644

5075-003 (Project)	Salary, Wages and Fringe Benefits	226,563
	Equipment	0
	Travel	10,250
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	124,548
	Consortium Costs	0
	Direct Costs	361,361
	Indirect Costs	415,696
TOTALS	Total Direct and Indirect Costs	777,057
5073-004 (Project)	Salary, Wages and Fringe Benefits	91,212
	Equipment	0
	Travel	4,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	54,788
	Consortium Costs	0
	Direct Costs	150,000
	Indirect Costs	78,000
TOTALS	Total Direct and Indirect Costs	228,000
5074-005 (Project)	Salary, Wages and Fringe Benefits	77,400
	Equipment	0
	Travel	0
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	70,550
	Consortium Costs	0

	Direct Costs	147,950
	Indirect Costs	82,112
TOTALS	Total Direct and Indirect Costs	230,062
5067-006 (Project)	Salary, Wages and Fringe Benefits	157,849
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	10,000
	Other Direct Costs (excluding Consortium)	128,351
	Consortium Costs	0
	Direct Costs	299,200
	Indirect Costs	216,900
TOTALS	Total Direct and Indirect Costs	516,100
5068-007 (Project)	Salary, Wages and Fringe Benefits	96,607
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	14,905
	Consortium Costs	0
	Direct Costs	114,512
	Indirect Costs	53,821
TOTALS	Total Direct and Indirect Costs	168,333
5065-008 (Project)	Salary, Wages and Fringe Benefits	210,394
	Equipment	0
	Travel	6,500

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	120,145
	Consortium Costs	0
	Direct Costs	337,039
	Indirect Costs	222,154
TOTALS	Total Direct and Indirect Costs	559,193
5066-009 (Project)	Salary, Wages and Fringe Benefits	175,272
	Equipment	0
	Travel	4,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	76,820
	Consortium Costs	0
	Direct Costs	256,092
	Indirect Costs	148,533
TOTALS	Total Direct and Indirect Costs	404,625
TOTALS		**7,112,904**

Categories Budget Summary

Categories	Components	Budget Period
R&R Budget - Senior/Key Person Funds Requested	5064-001 (Admin Core)	63,277
	5069-001 (Core)	47,472
	5070-002 (Core)	93,374
	5071-001 (Project)	11,314
	5072-002 (Project)	112,599
	5075-003 (Project)	20,906
	5073-004 (Project)	21,705
	5074-005 (Project)	36,320
	5067-006 (Project)	45,859
	5068-007 (Project)	58,762
	5065-008 (Project)	143,664
	5066-009 (Project)	42,237
TOTALS		697,489
R&R Budget - Other Personnel Funds Requested	5064-001 (Admin Core)	28,458
	5069-001 (Core)	184,320
	5070-002 (Core)	645,407
	5071-001 (Project)	73,110
	5072-002 (Project)	45,315
	5075-003 (Project)	205,657
	5073-004 (Project)	69,507
	5074-005 (Project)	41,080

	5067-006 (Project)	111,990
	5068-007 (Project)	37,845
	5065-008 (Project)	66,730
	5066-009 (Project)	133,035
TOTALS		1,642,454
R&R Budget - Section A & B. Total Salary, Wages and Fringe Benefits (A+B)	5064-001 (Admin Core)	91,735
	5069-001 (Core)	231,792
	5070-002 (Core)	738,781
	5071-001 (Project)	84,424
	5072-002 (Project)	157,914
	5075-003 (Project)	226,563
	5073-004 (Project)	91,212
	5074-005 (Project)	77,400
	5067-006 (Project)	157,849
	5068-007 (Project)	96,607
	5065-008 (Project)	210,394
	5066-009 (Project)	175,272
TOTALS		2,339,943
R&R Budget - Section C. Total Equipment	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0

	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Domestic Travel	5064-001 (Admin Core)	34,659
	5069-001 (Core)	2,563
	5070-002 (Core)	5,125
	5071-001 (Project)	3,000
	5072-002 (Project)	6,000
	5075-003 (Project)	10,250
	5073-004 (Project)	4,000
	5074-005 (Project)	0
	5067-006 (Project)	3,000
	5068-007 (Project)	3,000
	5065-008 (Project)	6,500
	5066-009 (Project)	4,000
TOTALS		82,097
R&R Budget - Foreign Travel	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0

	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Section D. Total Travel	5064-001 (Admin Core)	34,659
	5069-001 (Core)	2,563
	5070-002 (Core)	5,125
	5071-001 (Project)	3,000
	5072-002 (Project)	6,000
	5075-003 (Project)	10,250
	5073-004 (Project)	4,000
	5074-005 (Project)	0
	5067-006 (Project)	3,000
	5068-007 (Project)	3,000
	5065-008 (Project)	6,500
	5066-009 (Project)	4,000
TOTALS		82,097
R&R Budget - Tuition/Fees/Health Insurance	5064-001 (Admin Core)	0
	5069-001 (Core)	0

	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	10,000
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		10,000
R&R Budget - Stipends	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0

R&R Budget - Trainee Travel	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Subsistence	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0

	5066-009 (Project)	0
TOTALS		0
R&R Budget - Other Participants/Trainee Support Costs	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Section E. Total Participants/Trainee Support Costs	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	10,000

	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		10,000
R&R Budget - Materials and Supplies	5064-001 (Admin Core)	16,708
	5069-001 (Core)	85,999
	5070-002 (Core)	205,000
	5071-001 (Project)	63,946
	5072-002 (Project)	102,036
	5075-003 (Project)	116,348
	5073-004 (Project)	45,288
	5074-005 (Project)	30,550
	5067-006 (Project)	35,440
	5068-007 (Project)	8,000
	5065-008 (Project)	31,545
	5066-009 (Project)	62,820
TOTALS		803,680
R&R Budget - Publication Costs	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	2,000
	5072-002 (Project)	2,000
	5075-003 (Project)	0
	5073-004 (Project)	0

	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	1,500
	5065-008 (Project)	2,000
	5066-009 (Project)	4,000
TOTALS		11,500
R&R Budget - Consultant Services	5064-001 (Admin Core)	14,000
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		14,000
R&R Budget - ADP/Computer Services	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0

	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Subawards/Consortium/Contractual Costs	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Equipment or Facility Rental User Fees	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0

	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	5,405
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		5,405
R&R Budget - Alterations and Renovations	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	0
	5071-001 (Project)	0
	5072-002 (Project)	0
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	0
	5066-009 (Project)	0
TOTALS		0
R&R Budget - Other Direct Cost 1	5064-001 (Admin Core)	0

	5069-001 (Core)	53,280
	5070-002 (Core)	10,250
	5071-001 (Project)	2,500
	5072-002 (Project)	15,000
	5075-003 (Project)	8,200
	5073-004 (Project)	5,000
	5074-005 (Project)	40,000
	5067-006 (Project)	61,342
	5068-007 (Project)	0
	5065-008 (Project)	19,600
	5066-009 (Project)	10,000
TOTALS		225,172
R&R Budget - Other Direct Cost 2	5064-001 (Admin Core)	0
	5069-001 (Core)	135,300
	5070-002 (Core)	44,801
	5071-001 (Project)	2,000
	5072-002 (Project)	13,000
	5075-003 (Project)	0
	5073-004 (Project)	4,500
	5074-005 (Project)	0
	5067-006 (Project)	31,569
	5068-007 (Project)	0
	5065-008 (Project)	7,000
	5066-009 (Project)	0

TOTALS		238,170
R&R Budget - Other Direct Cost 3	5064-001 (Admin Core)	0
	5069-001 (Core)	0
	5070-002 (Core)	3,331
	5071-001 (Project)	3,000
	5072-002 (Project)	8,421
	5075-003 (Project)	0
	5073-004 (Project)	0
	5074-005 (Project)	0
	5067-006 (Project)	0
	5068-007 (Project)	0
	5065-008 (Project)	60,000
	5066-009 (Project)	0
TOTALS		74,752
R&R Budget - Section F. Total Other Direct Cost	5064-001 (Admin Core)	30,708
	5069-001 (Core)	274,579
	5070-002 (Core)	263,382
	5071-001 (Project)	73,446
	5072-002 (Project)	140,457
	5075-003 (Project)	124,548
	5073-004 (Project)	54,788
	5074-005 (Project)	70,550
	5067-006 (Project)	128,351
	5068-007 (Project)	14,905

	5065-008 (Project)	120,145
	5066-009 (Project)	76,820
TOTALS		1,372,679
R&R Budget - Section G. Total Direct Cost (A thru F)	5064-001 (Admin Core)	157,102
	5069-001 (Core)	508,934
	5070-002 (Core)	1,007,288
	5071-001 (Project)	160,870
	5072-002 (Project)	304,371
	5075-003 (Project)	361,361
	5073-004 (Project)	150,000
	5074-005 (Project)	147,950
	5067-006 (Project)	299,200
	5068-007 (Project)	114,512
	5065-008 (Project)	337,039
	5066-009 (Project)	256,092
TOTALS		3,804,719
R&R Budget - Section H. Indirect Costs	5064-001 (Admin Core)	73,837
	5069-001 (Core)	453,275
	5070-002 (Core)	1,321,127
	5071-001 (Project)	84,457
	5072-002 (Project)	158,273
	5075-003 (Project)	415,696
	5073-004 (Project)	78,000
	5074-005 (Project)	82,112

	5067-006 (Project)	216,900
	5068-007 (Project)	53,821
	5065-008 (Project)	222,154
	5066-009 (Project)	148,533
TOTALS		3,308,185
R&R Budget - Section I. Total Direct and Indirect Costs (G +H)	5064-001 (Admin Core)	230,939
	5069-001 (Core)	962,209
	5070-002 (Core)	2,328,415
	5071-001 (Project)	245,327
	5072-002 (Project)	462,644
	5075-003 (Project)	777,057
	5073-004 (Project)	228,000
	5074-005 (Project)	230,062
	5067-006 (Project)	516,100
	5068-007 (Project)	168,333
	5065-008 (Project)	559,193
	5066-009 (Project)	404,625
TOTALS		7,112,904

A. COMPONENT COVER PAGE

Project Title: Administrative Core - Core A

Component Project Lead Information:

Whitley, Richard J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The Administrative Core of the Antiviral Drug Discovery and Development Center (AD3C) will provide a key role in leadership, communication, coordination and oversight of the projects and cores, and stimulate collaboration and synergy between the projects. Operationally, it is in charge of fiscal and contractual management of the center and will plan and implement activities, such as meetings of the Executive Committee (EC), External Scientific Advisory Board (EAB), and an annual meeting of all projects and cores. In addition, it will manage the inter-institutional cooperative agreements. The core is also responsible for managing the solicitation and review of Supplemental Research Projects applications, such as those for additional product development and support for IND-enabling studies. Finally, the core will facilitate dissemination of progress and discoveries to the public. The broad objectives of the core are thus as follows:

1.Providing programmatic and administrative leadership
a.Make decisions, specifically “go versus no-go” decisions per discussions with the EC
b.Track and encourage research productivity
c.Promote interactions and collaboration between projects and cores, in particular to facilitate overarching synergy to pursue broad-spectrum antivirals
d.Monitor the direction and overall priorities of the Center
e.Directly interface with NIH staff

2.Fiscal and administrative management of the center
a.Finances: oversee expenditures, budget information, fiscal reports
b.Manage contracts and the consortium agreement
c.Establish and monitor compliance with federal and NIH regulations

3.Develop, support and monitor progress of projects
a.Manage projects by having regular conference calls and in-person meetings
b.Organize quarterly project reviews (face to face or teleconference) by the EC
c.Monitor overall Center research quality and progress annually by the EAB
d.Solicit additional regulatory guidance on an as-needed basis
e.Assist with identification and management of intellectual property developed by projects

4.Stimulate collaboration and synergy
a.Identify potential areas or topics of collaborations between projects and cores
b.Provide and facilitate access to resources needed in the projects
c.Ensure that active hits in one project with potential against other viruses are evaluated in other projects
d.Set up a data sharing and project tracking website

5.Facilitate meetings
a.Host monthly conference calls of project teams
b.Organize conference call-based and in-person meetings of the project and core PIs (EC)
c.Organize and implement Annual face to face meeting with all involved personnel, incl the EAB
d.Facilitate consulting and other scientific and professional meetings
e.Attend the annual CETR Program meeting and reverse site visit

6.Manage Supplemental Research Projects applications
a.Solicit proposals
b.Organize scientific and programmatic review of proposals

7.Outreach to the public
a.Set up and maintain a website
b.Write press releases
c.Publish newsletters/ebriefs
d.Help with scientific publications

We firmly believe that a strong Administrative Core will be crucial to the success of the AD3C. We have aggregated projects led by leading virologists in the country and are confident that the Administrative Core will facilitate communication and collaboration between the PIs and the cores. Accomplishing the objectives set out above will ensure focus and synergy among the projects, accelerating the development of new, potentially broad-spectrum therapeutics for (re-)emerging infections of flaviviruses, coronaviruses, alphaviruses and influenza.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Admin Core B2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the upcoming reporting period, Yr5 of the AD3C grant, the Admin Core plans to continue its leadership in ensuring the operations and activities of AD3C get executed as efficiently as possible. For this, it will focus on the following activities within the listed goals:

1.Providing programmatic and administrative leadership
a.We will continue our monthly conference calls with research updates of the Projects and Cores, with associated minutes and metric-tracking. The bi-weekly Core update meeting will also continue, as this has proven to be a very effective way of communicating action items and status reports for all of the Projects. In addition, small group meetings will continue be set up between the Projects and Cores to cover technical details that warrant a detailed discussion that does not need to include all members of the Executive Committee.

2.Fiscal and administrative management of the center
a.Core staff will continue to work with sites to ensure that they are aware of any federal regulations that are announced and related to the grant, particularly those that apply to sub-recipient awards. Financial accounts and invoice submissions will be tracked closely and budget updates will continue to be provided to sites on a regular basis as effort on the projects continues to advance.
b.As the end of Year 5 approaches, core staff will also work with all sites to ensure that they make preparations for completing Final financial and progress reports which will be due at the end of the next grant year.

3.Develop, support and monitor progress of projects
a.As mentioned under item 1, the monthly conference calls have proven to be very efficient in monitoring progress in the projects and cores. In addition, like in Year 4, we plan on having a mid-year face to face meeting with the EC members at an international meeting, possible the International Conference for Antiviral Research once again, to be held in 2018 in Porto, Portugal.

4.Stimulate collaboration and synergy
a.Cores B and C continue to maintain a database that can be used to share activities of all tested compounds against the various viruses and assays, to quickly identify compounds with broad-spectrum efficacy. In addition, in the monthly conference calls, areas of collaboration and materials of use to multiple projects continue to be routinely identified and offered by the personnel involved. Core C will continue to identify active molecules with an attractive enough profile to warrant testing in multiple virus families.

5.Facilitate meetings
a.The Admin Core will continue to host the monthly teleconference and ad hoc conferences to address specific concerns. In addition it will organize the mid-year EC face-to-face meeting at the ICAR meeting which is being held in Porto, Portugal. Finally, the core staff will organize the fifth annual AD3C meeting with all personnel involved, in Birmingham, AL. We will once again host the External Advisory Board during the annual AD3C meeting, to give them the most up-to-date information about the research progress prior to their evaluation of the program. We will continue to block sufficient time at the annual meeting to accommodate in depth detailed discussions between Project and Core personnel, in addition to high level presentations.

6.Manage Supplemental Research Projects applications
a.As the supplemental funding for the Zika Virus screen in Core B has been completed, there are no activities for this goal in Yr5.

7.Outreach to the public
a.The Admin Core will maintain and update www.uab.edu/medicine/ad3c as pertinent.

The major activities of Core A along with their specific objectives are described in bulleted form below, with a short description of the current status. Overall, Core A has met its objectives in Yr4.

1. **Providing programmatic and administrative leadership**
   a. Make decisions, specifically "go versus no-go" decisions per discussions with the EC

      We continued the practice of meeting with the EC and additional personnel on a monthly basis, via teleconference, to get an update on each of the projects and their interaction with the cores.
   b. Track and encourage research productivity

      We continued to distribute summarized minutes of the teleconferences mentioned above to the AD3C participants. Metrics such as publications and IP applications are actively being tracked; publications are listed on the website.
   c. Promote interactions and collaboration between projects and cores

      The projects and cores have been collaborating heavily, with interactions facilitated by the Administrative Core as well as initiated by project and core leaders and personnel themselves to discuss ad-hoc technical issues. Together with the Medicinal Chemistry Core, we are testing active lead compounds from one virus family across all the projects in this CETR, to find broad-spectrum therapeutics.
   d. Monitor the direction and overall priorities of the center

      The EAB met again in conjunction with the annual AD3C meeting and provided a report to the Administrative Core, attached to the overall CETR component of this progress report.
   e. Directly interface with NIH staff

      We continued to communicate with NIAID program staff on a regular basis and distributed pertinent information to the other research sites. The required annual reverse site visit has been scheduled for January 23, 2018.
2. **Fiscal and administrative management of the center**
   a. Finances: oversee expenditures, budget information, fiscal reports

      The Administrative Core has provided payment and tracking information to sites and provided guidance on current Year 4 budgeting as well as projections for Year 5. Earlier this year, the University of Alabama system conducted an internal financial audit of the grant account which resulted in "No Findings" , reporting that PI and staff were in full compliance of policy and procedures.
   b. Manage contracts and the consortium agreement

      The Administrative Core continues to make sure the agreement between Gilead and the Consortium gets updated as novel compounds enter the consortium.
   c. Establish and monitor compliance with federal and NIH regulations

      The Administrative Core staff continues to maintain a contact list which includes key research, administrative and financial personnel, and is in regular communication with them regarding all AD3C activities and regulatory and financial requirements under the award. The Business Officer continues to work closely with UAB's Office of Sponsored Program and Grant Accounting as well as with similar offices in the participating institutions to ensure submission of required documents and compliance with federal policies.
3. **Develop, support and monitor progress of projects**
   a. Manage projects by having regular conference calls and in-person meetings

      As noted above, project teams and the EC had monthly conference calls with progress being tracked and to-do-items clearly delineated by preparing and distributing minutes by the Associate Director. In addition, there is an in-person meeting every 2 weeks between all Cores at Southern Research, along with Investigators from Project 4.
   b. Organize quarterly project reviews (face-to-face or teleconference) by the EC

      At this point, the monthly update meetings serve the goal of informing the EC of progress and obtaining their input on decisions to be made.
   c. Monitor overall Center research quality and progress annually by the EAB

      The EAB met for the fourth time at the annual meeting in October 2017 and subsequently provided a report with advice at a high, project portfolio level. Last year's additions to the EAB, Kara Carter, PhD, and Pei-Yong Shi, PhD, continue to provide a translational perspective on activities of AD3C.
   d. Solicit additional regulatory guidance on an as-needed basis

As we are getting closer in certain projects towards animal models, we received regulatory guidance from the EAB to forego non-human primate studies; rather, show proof of concept in a rodent model, and then move into human studies as soon as possible.

e. Assist with identification and management of Intellectual Property developed by projects

As described in the consortium agreement, novel chemical scaffolds with potent antiviral activity will be protected, using Southern Research as the lead institution since they have the most expertise in this area. We have filed one patent application in this project period.

4. Stimulate collaboration and synergy

a. Identify potential areas or topics of collaborations between projects and cores

As mentioned earlier, a set of about 30 compounds has been identified to be attractive enough to be tested across all projects, to identify broad spectrum antivirals. For at least one of these compounds, the mechanism of action is being investigated by several project sites, to confirm and compare resulting potential target proteins.

b. Provide and facilitate access to resources needed in the projects

The Admin Core facilitated budgeting for the final year of the grant. One project requested rebudgeting of available funds during this current year to cover needed equipment costs.

c. Ensure that active hits in one project with potential against other viruses are evaluated in other projects

As mentioned earlier, this is being actively pursued, under joint leadership with Core C

d. Set up a data sharing and project tracking website

We continue to use the Enterprise Content Management software “Documentum CenterStage”. All the AD3C and EAB members have access to this secured site via a login name and password. Core C maintains a specialized database with more advanced tools to query structures, antiviral activity and many other parameters of the compounds.

5. Facilitate meetings

a. Host monthly conference calls of project teams

An audio and web meeting service available through AT&T continues to be utilized by Administrative Core personnel to host the monthly project team meetings.

b. Organize conference call-based and in-person meetings of the project and core PIs (EC)

In addition to getting together at the annual AD3C meeting in October, the EC met in May 2017, in conjunction with the International Conference for Antiviral Research (ICAR) in Atlanta, GA.

c. Organize and implement Annual face to face meeting with all involved personnel, including the EAB

The Admin Core has hosted AD3C’s fourth annual scientific meeting, in Birmingham, AL, on October 26-27, 2017, to discuss the status of each of the projects. Three of the four EAB members attended. Small group meetings were used to cover technical details of screening protocols and chemistry directions.

d. Facilitate consulting and other scientific and professional meetings

The projects are in an early phase of discovery and no consulting other than that received from the EAB has been required as of yet.

e. Attend the annual CETR Program meeting and reverse site visit

As noted above, a reverse site visit has been scheduled with NIAID staff for January 23, 2018. With no CETR Program planned, ICAR once again served as a national meeting site.

6. Manage Supplemental Research Projects applications

a. The Admin Core facilitated a request for proposals for supplemental research funding in the Spring of 2016; 7 applications were received from 5 different institutions, all of which were reviewed by at least 3 reviewers in the EC. The EAB received these reviews and ultimately selected 2 applications to be forwarded to NIAID for consideration; none were selected for funding.

b. The final segment of supplemental Zika funding that was provided by NIAID was awarded to SR which completed screening in this project period.

7. Outreach to the public

a. Set up and maintain a website

The Administrative Core maintains a website: www.uab.edu/medicine/ad3c, which contains descriptions of the Center, projects and cores and associated personnel, along with a publication list.

b. Write press releases

No press releases were required in this project period.

c. Publish newsletters/ebriefs

The Administrative Core has not had a need yet to publish an electronic “ebrief”.

d. Help with scientific publications

The Admin Core continues to ensure acknowledgement of grant support and submission to PubMed Central and PMCID requirements.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Admin Core-5064

RESEARCH & RELATED BUDGET - SECTION A & B **FINAL**

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Richard	J	Whitley		Project Leader	(b)(4); (b)(6)				18,700.00	5,647.00	24,347.00
2.		Maaike		Everts		Co-Investigator					29,900.00	9,030.00	38,930.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **63,277.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	1 Program Manager, 1 Program Coordinator	(b)(4)			21,018.00	7,440.00	28,458.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**28,458.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**91,735.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	34,659.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**34,659.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,708.00
2. Publication Costs	0.00
3. Consultant Services	14,000.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**30,708.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**157,102.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	157,102.00	73,837.00
		Total Indirect Costs	**73,837.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Shon Turner 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**230,939.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Admin Core Budget Justification Yr 5 12-2017 final.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel

Richard J. Whitley, MD, PD/PI, (b)(4) months: Dr. Whitley will continue to serve as the Program Director/Principal Investigator of AD3C, and as Director of the Administrative Core. Dr. Whitley is currently Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, and holds the Loeb Chair in Pediatrics in the School of Medicine at the University of Alabama at Birmingham (UAB). He will continue to manage and provide guidance and oversight to the AD3C investigators, communicate regularly with the NIAID Program Officer, members of the EC and Scientific Advisory Board, and work closely with the Associate Administrator and staff to ensure that planned project milestones are met. He will also continue to moderate monthly teleconferences, attend the NIH reverse site visit and lead the AD3C Center annual meeting.

Maaike Everts, PhD - Associate Administrative Director (b)(4) months,
Dr. Everts is an Associate Professor in the Department of Pediatrics, School of Medicine at the University of Alabama at Birmingham. She continues to serve as the Associate Director of the Alabama Drug Discovery Alliance. She will continue to lead day to day research efforts of the AD3C, facilitating communication and interaction between project investigators and the cores, and serve as primary liaison between projects, providing research updates to the Director, Executive Committee and Scientific Advisory Board. She will also continue to work closely with the Program Director and Administrative Core staff to monitor the status of all projects and cores with respect to administrative, financial and regulatory aspects of the program.

Mary Wyatt Bowers, MA, (b)(4) months: Ms. Bowers will continue to oversee the financial administration of AD3C, and maintain responsibility for budgetary issues and invoicing, and coordinate with Sponsored Programs on subawards, and amendments. She will continue to aid Maaike Everts (see above) with meeting organization and planning, and assist with the activities related to the final year reports and closeout of the current grant. She also serves as the liaison with the UAB Office of Sponsored Programs, Grant Accounting, and Principal Investigators and institutions for matters related to financial and contractual agreements.

Sara Davis, (b)(4) months, Ms. Davis is the program coordinator and administrative assistant to Dr. Rich Whitley. She will continue to assist Drs. Whitley and Everts with logistical aspects of meeting scheduling and organization as well as communications with external institutions and agencies and report preparations.

Consultants

Funds are budgeted for four members of the required external scientific advisory board who are expected to continue their attendance at the annual AD3C meeting and review activities of all projects. One member also attends the NIH site visit. As projects enter the final year of the grant, their continued guidance, oversight, and recommendations for the four projects remains of critical importance. The budget is based on estimated $3,500 reimbursement per advisor annually.

Supplies

Minimal funding is requested to provide for copier, postage, and office supplies needed to manage administrative activities of the large multi project program. Costs are estimated based on historical experience with similar multi-site project management.

Travel

Funds are requested for the following travel:

- Annual NIH reverse site visit expenses for the PD/PI, 1-2 project leaders, EAB member and administrative staff in Rockville, MD.

- PD/PI, core personnel and/or Administrative Associate Director travel to provide technical assistance or oversight to one or more projects ($3,000)
- Executive committee meeting for PD/PIs and project leaders held approximately six months after Center Annual meeting.
- AD3C Center annual meeting held in Birmingham that includes PD/PIs, project leaders, postdocs, scientific advisory board members.
- Travel for project leaders and investigators to attend an annual research conference similar to the originally planned national CETR meeting. This past year, AD3C investigators arranged for breakout sessions and met during the International Conference on Antiviral Research (ICAR) which was held in Atlanta, Georgia on May 21-25, 2017. This served as a mid-year meeting for the grant researchers.

Other Expenses

Teleconference/web meeting: Funds will continue to be used to cover the costs of monthly teleconferences and/or web meetings outlined in the administrative core plan to review and monitor projects and update project leaders on a continuous basis.

Computer maintenance: costs include website updates to allow for communication and data sharing among investigators as well as providing a means for public access.

Publication/duplication costs: Funding is requested to cover costs related to publications and copying of draft progress and final reports, meeting minutes, and material used for annual or executive committee meetings. Costs are based on historical costs for meeting and reports used for a similar program.

Annual meeting expenses: funds are included to cover costs related to hosting the large Center annual meeting for investigators and scientific advisors for two days. The budget includes costs for meeting space, audiovisual services, incidental refreshments and meals for attendees, and rental of items such as poster display boards.

A. COMPONENT COVER PAGE

Project Title: Project 1.1 Identification and Development of Anti-Flavivirus Lead Drug Candidates

Component Project Lead Information:

NELSON, JAY A

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The flavivirus genus comprises >70 single stranded, positive sense RNA viruses that are associated with significant worldwide morbidity and mortality. Flaviviruses, which are primarily insect-borne, have been found on every inhabited continent (7). Unfortunately, current therapeutic options for treating diseases associated with these viruses are limited. This proposal builds on existing expertise in small molecule screening for DENV and is designed to identify small molecule compounds with the potential to be developed as antiviral agents. The initial screen in this proposal will focus on two medically relevant flaviviruses: dengue viruses (DENV) and West Nile virus (WNV). An existing screening platform will be adapted to screen multiple compound libraries, which include a high representation of nucleoside and nucleotide analogs, potentially compounds that have activity against multiple flaviviruses. If broad-spectrum leads with efficacy against multiple viruses can be identified, their further development will be emphasized. In order to enrich for potentially broadly acting compounds, we will focus on compounds that target one of the following important enzymatic activities of the flavivirus NS5 protein: the RNA-dependent RNA polymerase (RdRp), which is essential for replication of the viral RNA genome and the 2'-O-methyltransferase (2'-O-MTase), which is required for the virus to evade the host innate immune response. These activities are conserved among the flaviviruses, and similar activities are found in other virus families as well. The overall CETR proposal contains several projects focused on various virus families that are linked by a central screening facility and compound libraries. Therefore, the parallel screening strategies will maximize the likelihood of identifying broad-spectrum antiviral agents that may function across multiple virus families. The specific aims of Project 1 are:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds identify novel inhibitors of flavivirus replication.

Rationale: The Southern Research Screening Core (SR SC) has developed and validated cell-based, high-throughput assays for inhibitors of DENV and WNV induced cytotoxicity. Initial use of this, or similar, assays has already identified several compounds with antiviral activity. Therefore, this assay will be used to screen novel libraries that have not previously been extensively screened against human pathogens.
Experimental strategy: A CPE based assay will be used as a primary screen for compounds with anti-DENV or anti-WNV activity. Additionally, the WNV screen will be modified in order to allow the detection of compounds that inhibit the viral 2'-O-MTase, thereby sensitizing the virus to the actions of interferon and its effectors. Following the initial screen, "hits" will be evaluated in dose response and cytotoxicity assays in order to determine EC50, CC50, and selective indexes.

Aim 2: Characterize the antiviral activity of hit compounds

Rationale: Hit compounds will be further characterized with regard to efficacy and mechanism of action. The primary screen will potentially identify compounds that inhibit any of the stages of the viral replication cycle, therefore, secondary experiments are designed to elucidate the stage at which individual compounds act. Additionally, we will also characterize the compounds with regard to breadth of activity against other viruses, and examine the potential for evolution of compound-resistant mutants.
Experimental strategy: We will initially test compounds against sub-genomic viral replicons, which will identify compounds that do not function through affecting viral entry or egress, allowing us to focus on inhibitors of translation, protein processing, or RNA replication. We will also identify compounds that function through inhibition of the 2'O MTase, as well as compounds that act non-specifically through induction of interferon or other innate pathways. Compounds will also be evaluated in viral growth assays in order to evaluate the their effect on inhibition of production of infectious progeny virus. Additionally, we will analyze compound effects against multiple viruses and in multiple cell types. Finally, we will test the ability of the virus to develop resistance to individual compounds, as well as characterize any such mutants.

Aim 3: Chemical optimization and in vivo efficacy of lead compounds in animal models of West Nile and Dengue infection.

Rationale: Hit compounds identified and characterized above will be optimized to increase efficacy, selectivity, and bioavailability. These compounds will progress to testing in mouse models of infection.
Experimental strategy: Specific compounds and scaffolds will be triaged by the Medicinal Chemistry and Lead Development Core (MCLDC). Compounds with appropriate pharmacokinetic properties will be tested for prophylactic and therapeutic effects in mouse models of WNV and DENV infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 1.1 B.2 Accomplishments Jay Nelson.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

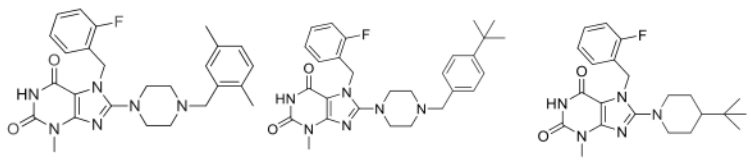
B.6. Plans for next year. Mechanism of action studies on selected active compounds will continue. In addition to sequencing of resistant mutants, we will continue to employ a series of assays to determine the stage of the viral replication cycle that is targeted by the individual compounds (some of which have been completed for SRI-37776). Time-of-addition studies will be used to determine when the drug must be present to affect inhibition. Results of these studies will suggest if the effect is primarily on viral entry or a later step. We will use a sub-genomic replicon to examine the effect on viral protein expression and RNA replication. We will also assay production of infectious progeny by plasmid expression of the viral C, prM, and E proteins in cells expressing a reporter (GFP) replicon. The structural proteins are capable of packaging the replicon and are released as infectious particles that can transfer the replicon (and GFP+ phenotype) to naïve cells. This assay can be used to assess the effect of compounds on assembly and egress of viral particles, as well as changes in the infectivity of progeny virus. We will additionally employ assays of viral attachment and entry if the time-of addition experiments suggest that the drug acts early in infection. These assays are currently established in our lab (PMID: 24599995). Following completion of pharmacokinetic analysis of lead compounds, we expect that we will begin testing of lead compounds in animal models of infection. For analysis of DENV replication, these studies will be carried out in interferon-deficient AG129 mice.

B. 2 Accomplished under goals:

Major activities and objectives: The major activities for this reporting period have been: Follow-up on completed HTS for small molecule inhibitors of West Nile virus (WNV) in the presence or absence of the antiviral cellular protein IFIT1 conducted by Core B, and continuing follow-up and characterization of compounds and compound analogs with anti-DENV and anti-WNV activity.

Results and outcomes:

WNV inhibitors: Core B has completed the HTS screen for WNV inhibitors. This screen was carried out in matched cell lines in the presence or absence of IFIT1 (expressed via an inducible promoter). As detailed in the initial proposal, inhibitors that are dependent on the presence of IFIT1 are potentially acting against the viral 2'-O-methyltransferase (2'O-MTase). IFIT1-dependent compounds are undergoing secondary screens in the Diamond lab (Washington Univ), while inhibitors that act in the presence or absence of IFIT1 will be re-screened in the Nelson/ Hirsch labs (OHSU).

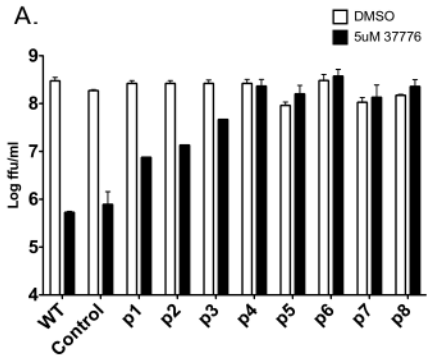


SRI-37776
EC_{90}=0.7 µM
VTR=3.2 @ 10 µM
CC_{50}=12.4
Sol=9.7 µM
HLM $t_{1/2}$=4.5 min
MLM $t_{1/2}$=8.7 min

SRI-38841
EC_{90}=0.87 µM
VTR=3.5 @ 10 µM
CC_{50}= >30
Sol= 1.8 µM
HLM $t_{1/2}$=20.9 min

SRI-38840
EC_{90}=0.88 µM
VTR=3.5 @ 10 µM
CC_{50}= >22
Sol= 1.9 µM
HLM $t_{1/2}$=64.7 min

Fig. 1 Structures and properties of SRI-37776, -38841, and -38840



B.

Virus	IC90
WT	1.2uM
Control	3uM
p1	0.5uM
p2	0.6uM
p3	3.2uM
p4	>30uM
p5	>30uM
p6	>30uM
p7	>30uM
p8	>30uM

Fig 2. Elicitation of SRI-37776 resistant WNV. WNV was grown in the presence of 5 µM SRI-37776 for indicated number of passages. **A.** Growth of supernatant virus following each passage is shown in the absence or presence of drug (48 hpi). **B.** For each passage, an IC_{90} for SRI-37776 was determined.

Lead compound SRI-00037776: Compound SRI-00037776 was one lead that inhibits WNV replication with or without the presence of IFIT1. Further analysis, however, suggested several "red flags" requiring further chemical optimization. Analog compounds SRI-38841 and SRI-38840 had more favorable *in vitro* toxicity (≥30 and 22 µM, respectively) as well as improved *in vitro* ADME profiles compared to the parent compound. (Fig. 1).

Through passage of WNV (NY99) in the presence of 5 µM SRI-37776, we were able to elicit resistant variants of the virus. (Fig. 2). Following 4 passages in the presence of compound, resultant virus was unaffected by drug concentrations up to 30 µM. Virus passaged in parallel without compound ('Control') remained sensitive, and SRI-37776 maintained an IC90 in the low micromolar range. Sequencing of resistant viruses revealed three non-synonymous mutations not present in virus passed in the absence of drug. These mutations (NS1 T283A, NS4B V88A, and NS5 K193R) are currently being engineered into a WNV infectious clone for further analysis of how they impact resistance to the compound. We are also repeating the passage of virus under SRI-37776, as well as in the presence of the analog, SRI-38841, selection in order to determine if identical, or novel, mutations are found in resistant mutants.

Time-of-addition studies were performed with SRI-37776 to determine if drug needs to be present at the beginning of the infectious cycle or if it is capable of effecting inhibition of replication when added later. Cells were infected (MOI=5) and drug added to 5 µM final concentration at indicated times post infection. At 24 hpi, cells were washed and refed with medium + drug, followed by harvest at 48 hpi and quantification of supernatant virus by focus-forming assay. As shown (Fig. 3) SRI-37776 is effective when added through 24 hpi, demonstrating that the mechanism of inhibition does not involve entry or early events in replication.

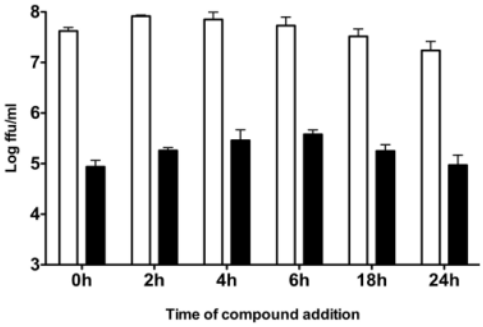


Fig 3. SRI-37776 is effective throughout the WNV replication cycle. HEK293 cells were infected with WNV (MOI=5) at t=0. 5 µM SRI-37776 (black bars) or DMSO (white bars) was added at the indicated time pi. At 24 hpi, medium was removed from cells, followed by wash and replacement with fresh medium

To examine the effect of SRI-37776 on viral RNA replication, cells were treated with 5 μM drug or vehicle (DMSO) control. Total RNA was isolated from cells at indicated times and WNV RNA quantified by qRT-PCR (Fig. 4). As shown, intracellular WNV RNA was significantly reduced in drug treated cells, suggesting that SRI-37776 acts on viral replication, protein expression, or establishment of the replication complex.

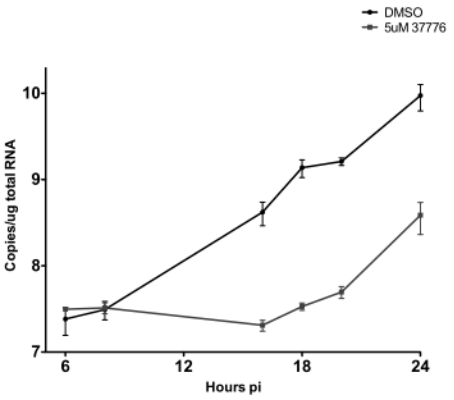


Fig 4. SRI-37776 inhibits WNV RNA replication. HEK293 cells were infected with WNV (MOI=5) and treated with 5 μM SRI-37776 (red) or DMSO (black).Cells were lysed at indicated times and total RNA isolated. WNV genomes were quantified by qRT-PCR.

Analysis of compounds from supplemental Zika virus (ZIKV) screen: Southern Research has completed an additional high throughput screen for inhibitors of ZIKV via supplemental funding of the NIAID. Compounds identified as active in this screen are undergoing investigation for activity against dengue virus (DENV) and WNV.

Other flavivirus inhibitory compounds: Additional series of WNV inhibitory compounds based on compounds SRI- 37710 (IC_{90}: 4 μM, log10 viral titer reduction at 10 μM (VTR): 2) and SRI-22808 (IC90: 0.9 μM, VTR: 4.1) are currently being analyzed. Further analysis of DENV-inhibitory compounds is also ongoing.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Jay		Nelson		Project Leader	(b)(4); (b)(6)				27,765.00	5,553.00	33,318.00
2.		Alec		Hirsch		Co-Investigator					32,082.00	8,983.00	41,065.00
3.		Jessica		Smith		Co-Investigator					47,780.00	21,501.00	69,281.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **143,664.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	1 Staff Scientist,1 Sr. Res.Assoc, 1 Lab Aide	(b)(4)			50,136.00	16,594.00	66,730.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**66,730.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**210,394.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Oregon Health and Science University

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	31,545.00
2. Publication Costs	2,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Charges, lease and per diem fees	19,600.00
9. Microscopy, sequencing	7,000.00
10. M-Chem Core Service	60,000.00
Total Other Direct Costs	**120,145.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**337,039.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	75.0	274,539.00	205,904.00
2. MTDC	26.0	62,500.00	16,250.00
		Total Indirect Costs	**222,154.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, Arif M. Karim, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**559,193.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Nelson_Whitley_Project1_Budget_Justification_Year 5.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION, YEAR 5:

Nelson- Anti-flavivirus drug discovery

PERSONNEL:

Jay Nelson, Ph.D., Principal Investigator: (b)(4) months (b)(4) Dr. Nelson is a senior molecular virologist with over 180 papers and reviews on a variety of topics, including herpesviruses, retroviruses, and flaviviruses. The primary focus of Dr. Nelson's research over the years has centered on the molecular pathogenesis and immune response to viruses including herpesviruses, flaviviruses and retroviruses. Dr. Nelson's group has used molecular and animal model approaches over the past 30 years to characterize cytomegalovirus (CMV) and flavivirus replication. Dr. Nelson's group, in collaboration with Klaus Früh and Alec Hirsch, used functional genomic approaches to determine that cYes, a cellular Src kinase, is an important regulator of flavivirus maturation. We have also shown that capsid interaction with cYes alters tight junction function by targeting degradation of Claudin 1 by the lysosome and have shown that WNV regulates the unfolded protein response (UPR) through CHOP to block cellular apoptosis. This project is the result of a long-term collaboration between Drs. Früh and Hirsch to identify potential lead compounds that inhibit Dengue and WNV replication. Dr. Nelson will be responsible for the planning of experiments and oversight on progress for this grant, as well as for communication with other Project and Core leaders and dissemination of results.

Alec Hirsch, Ph.D., Co-Investigator: (b)(4) months, (b)(4) Dr. Hirsch is an Assistant Scientist at the Vaccine and Gene Therapy Institute (VGTI) and will serve as Co-Investigator of the anti-flavivirus drug discovery project. Dr Hirsch has extensive experience with *in vitro* and *in vivo* models of flavivirus infection. He will be responsible for directing the investigation of the efficacy of compounds *in vitro* as well as *in vivo* in mouse models of viral infection. His duties will include coordination with other arms of this proposal, disseminating data sets produced during this project, and ensuring timely completion of the proposed work.

Jessica Smith, Ph.D., Co-Investigator: (b)(4) months, (b)(4) Dr. Smith received her Ph.D. in biomedical sciences from University of New Mexico School of Medicine in 2008, where she studied entry and trafficking of human papilloma virus. Since her time at the VGTI she has studied multiple aspects of flavivirus-host cell interactions, including identification and characterization of anti-flaviviral compounds. She will be responsible for directing lab personnel in conducting secondary and tertiary screens in conjunction with Dr. Hirsch, as well as supervising follow-up studies characterizing mechanisms of compound action. Additionally, she will be responsible for organizing and distributing data to other projects within the A3DC.

Meaghan Hancock, Ph.D., Staff Scientist 3: (b)(4) months; (b)(4) Dr. Hancock is a molecular virologist working with Dr. Nelson the VGTI. She will be responsible for conducting secondary and tertiary screens as well as conducting follow-up studies characterizing mechanisms of compound action. Dr. Hancock will also assist in the *in vivo* studies to be conducted in later years of this project.

Christopher Parkins, M.S., Senior Research Associate: (b)(4) months, (b)(4) Mr. Parkins will be responsible for producing WNV and DENV titered stocks for infection studies and assisting as needed with *in vitro* assays. Mr. Parkins will also be responsible for management of the AG129 mouse colony, infection of mice for *in vivo* studies, processing of animal samples, performing quantitative RT-PCR detection of virus in plasma and tissue samples from infected mice.

Erika Ferreira-Martine, Lab Aide: (b)(4) **months,** (b)(4) Ms. Ferreira-Martine will wash, organize and track all lab supplies for this project.

SUPPLIES:

Plasticware ($5,545/ yr Yr 1-5)

Disposable plasticware will be required for cell and virus culture, DENV and WNV titration and virus isolation, and molecular biological work. This includes tissue culture dishes of myriad sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage.

Tissue Culture Supplies ($6,000/ yr Yr 1-5)
These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes.

qRT-PCR (Taqman) ($10,000/ yr Yr 1-5)
qRT-PCR will be used for the detection of both WNV and DENV in animal tissues and quantitation of viral RNA replication in culture. Reagents for virus detection include: Reverse transcription reagents, ABI Master mix containing Taq polymerase, virus-specific primers and TaqMan probes, 96-well optical plates and caps.

Surgical Supplies: ($2,500/ yr Yr 1-5)
Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs required for obtaining blood samples and tissues, isoflurane for anesthesia; Alzet osmotic pumps for cases in which continuous delivery of compounds is to be examined.

Enzymes/ molecular biology supplies/ chemicals: ($5,000/ yr Yr 1-5)
PCR reagents for cloning of viral mutants, restriction enzymes, Western blotting and protein analysis supplies, buffers, acrylamide, agarose.

Toxicity assays: ($2,500/ yr Yr 1-5)
Celltiter Glo reagent (Promega) for determination of toxicity of individual compounds.

Mice purchase ($6,000/ yr Yr 1-5)
We expect that we will require approximately 350 of each strain for the experiments described in this proposal. We will maintain a colony of AG129 mice to provide mice for DENV experiments. Calculation of number of breeding cages and cages to maintain weaned mice to support proposed experiments (according to “Breeding Strategies for Maintaining Colonies of Laboratory Mice.” Published 2007, The Jackson Laboratory) = 20 cages. We will purchase C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories ($16.40/animal) = approx. $6,000.

TRAVEL:
$6,500/year for Co-Investigators to attend an international meeting pertaining to antiviral therapeutics and vaccines directed against RNA virus infection and disease.

OTHER EXPENSES:
Mice per diem ($13,600/ yr Yr 1-5)
We expect that we will require approximately 350 of each strain for the experiments described in this proposal. We will maintain a colony of AG129 mice to provide mice for DENV experiments. Calculation of number of breeding cages and cages to maintain weaned mice to support proposed experiments (according to “Breeding Strategies for Maintaining Colonies of Laboratory Mice.” Published 2007, The Jackson Laboratory) = 20 cages. At $1/cage/day per diem = $7,300 annually. Cage costs for weaned mice and housing during experiments at $3.50/cage/day per diem (approx. 2 months per cage) = $4,200. We will purchase C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories. Per diem cage costs for these mice at $3.50/cage/day per diem should total $2,100 (assuming a total of 1 month housing).

Microscopy: ($2,000/ yr Yr 1-5);
Quantitative fluorescence microscopy will be used in secondary screens to evaluate compound efficacy. Immunofluorescent staining will be performed at VGTI and plates read by the automated fluorescence microscope at the Oregon Translational Research and Drug Development Institute (OTRADI). Cost is $50 for setup and $125/hour + 35% overhead.

Sequencing: ($5,000/ yr Yr 1-5)
At $500/sample, we will use deep sequencing to identify resistance mutations that arise due to compound treatment. We will sequence approximately 5 samples per year including both WNV and DENV isolates.

Publications ($2,000/ yr Yr 1-5)
For publication costs. We estimate 1-2 publications per year.

M-Chem Core Services ($60,000/ yr Yr 2-5)
M-Chem Core Services: ($60,000/yr, Years 2–5): Dr. Aaron Nilsen is Director of the OHSU Medicinal Chemistry Core facility (M-Chem Core). For this project, the M-Chem Core will design chemical biology experiments to help biological researchers investigate the mechanisms of action of small molecule antivirals. Additionally, the Core will synthesize analogs of small molecule antivirals for use in mechanism of action experiments. In terms of experience, the Core Director has more than 17 years of experience in chemical biology, medicinal chemistry, drug discovery and organic synthesis. Dr. Nilsen was the lead synthetic chemist on the multinational Medicines for Malaria Venture team that recently delivered a new quinolone-3-diarylether compound (ELQ-300) to the MMV for clinical development. Chemical reagents will be required to synthesize analogs including building blocks and solvents, which will be charged through the Core.

INDIRECT COSTS:
The bulk of the indirect costs ($205,904) are calculated at the rate of 75% (based on direct costs of $274,539) for work to be conducted at the OHSU West Campus. The remainder of the indirect costs ($16,250) are calculated at an F&A rate of 26%, which is the off-campus rate used for research projects at OHSU. It is OHSU's policy not to charge a significantly higher indirect cost rate on projects that transfer from outside entities, even though the work is done on-campus. Charging the full rate on this project would create a financial hardship in terms of achieving and completing the aims of the project. Therefore, a 26% modified total direct cost rate is used for that portion of the project ($62,500 direct costs: $60,000 for M-Chem core serves and $2,500 for travel) that will be conducted at the OHSU Medicinal Chemistry Core facility (transferred from the Portland VA Research Foundation, Inc., which is outside OHSU).

A. COMPONENT COVER PAGE

Project Title: Project 2.1 Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics

Component Project Lead Information:

(b)(6); (b)(3):7 U.S.C. § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication.

Aim 2. To identify and develop inhibitors of CoV RNA capping activity.

Aim 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 2 B2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Project 2 B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for the next reporting period are based on continuation of experiments described above. Briefly, SR leads will be assessed for activity against MHV, other human CoVs, and pre-emergent bat CoVs. We will proceed to probe mechanisms of action by passage for resistance and identification of target and mutations (VUMC). Promising results will trigger an evaluation of in vivo efficacy using murine models of CoV pathogenesis (UNC). We will initiate studies of combinatorial activity profiles and interactions of remdesivir, SR anti-CoV lead compounds, and EIDD-1931. These efforts are designed to enhance activity of, prevent resistance to, and broaden the spectrum of anti-CoV pipeline drugs identified during the preceding four years of U19 support and are distinct from any follow on funding obtained.

B.2. What was accomplished under these goals?

B.2.a. Major activities, specific objectives, significant results, and key outcomes. Our collaborations with Gilead Sciences (GS) and Southern Research (SR) have been productive in preclinical development of candidate therapeutics against SARS-CoV, MERS-CoV and potentially pre-pandemic zoonotic CoVs. An additional candidate lead has been identified.

Remdesivir (Gilead GS-5734). During Year 4 of funding, we published a suite of data in Science Translational Medicine demonstrating that the nucleotide prodrug, remdesivir (GS-5734), can inhibit multiple genetically distinct human and zoonotic CoV (Sheahan et al. 2017. PMC5567817). Importantly, replication of SARS-CoV and MERS-CoV was inhibited in primary human airway epithelial (HAE) cell cultures with submicromolar EC_{50} values. Replication of bat CoVs, prepandemic bat CoVs, and circulating contemporary human CoV were also inhibited, thus demonstrating broad-spectrum anti-CoV activity. In a mouse model of SARS-CoV pathogenesis, both prophylactic and therapeutic administration of remdesivir significantly improved outcomes, reduced lung viral load and improved respiratory function. Recently, the Baric laboratory has demonstrated that both prophylactic and therapeutic remdesivir significantly improved outcomes in MERS-CoV infected mice with improved pulmonary function and reduced viral loads. These data are key for moving remdesivir towards licensure and provide substantive evidence that remdesivir may prove effective against human MERS-CoV infection, circulating human CoV and emerging CoV of the future.

To define remdesivir mechanisms of action against CoVs and potential for viral resistance, we passaged the β-CoV murine hepatitis virus (MHV) in the presence of the remdesivir parent nucleoside GS-441524. Two mutations, F476L and V553L, conferring up to 5.6-fold resistance to remdesivir as determined by EC_{50}, were selected in the nsp12-RdRp (**Fig. 1).** These residues are conserved across all CoVs. Resistant viruses were unable to compete with WT in direct co-infection passage in the absence of remdesivir. Introduction of the MHV resistance mutations into SARS-CoV resulted in the same *in vitro* resistance phenotype, and also attenuated SARS-CoV pathogenesis in a mouse model. Finally, we found that an MHV mutant lacking nsp14-exoribonuclease (ExoN) proofreading was significantly more sensitive to remdesivir. Combined, the results indicate that remdesivir interferes with the RdRp even in the setting of ExoN proofreading activity. While partial resistance was achieved with passage in the presence of remdesivir, resistance was attained at the cost of replicative fitness and pathogenic potential. These studies further support development of remdesivir as an antiviral targeting CoV. This work has been submitted to **mBio** (Agostini, Andres, et al. submitted). The work has resulted in this year a published study in Science translational medicine and a submitted report to mBio (under review Dec 2017). In addition two sources of follow-on funding have been identified

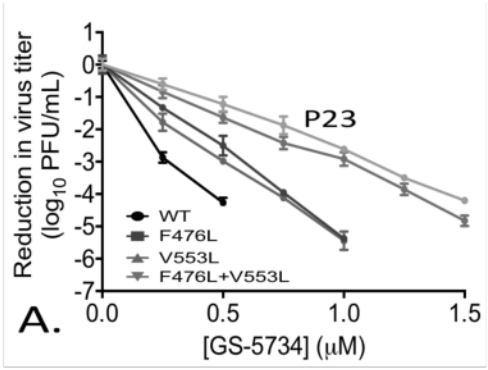


Fig. 1. Mutations in the MHV nsp12-RdRp (F476L and V553L), selected by GS-5734 passage, decrease virus sensitivity to GS-5734 (**A**), but increase sensitivity to cytidine analog EIDD-1931 (**B**). Shown in both is log reduction in titer normalized to 0. P23 (orange) indicates the GS-5734 passage 23 virus. Mutations were identified and introduced in isogenic MHV cloned virus.

Remdesivir (GS-5734). Studies with remdesivir resulted new R01 via the RFA: Partnerships for Countermeasures Against Select Pathogens [RFA-AI-16-034]. The partnership of UNC, VUMC, Gilead, and UTMB will move remdesivir through preclinical development toward IND submission. ***Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV*** (R01AI132178, 8/9/17-8/8/22, P.I. Ralph Baric, Tim Sheahan).

Mechanisms of Resistance of CoVs to nucleoside analogs (b)(6); (b)(3):7 U.S.C. § 8401 (Vanderbilt) has performed significant studies (paper submitted) of remdesivir activity and resistance, (b)(6); received F31 support to continue these studies with remdesivir. (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

SR compounds. Southern Research Institute (SR) compounds SR-36097 and SR-36100 were positive in a SR high-throughput screen (HTS) of >200K small molecules for compounds that inhibited SARS-CoV replication based on CPE reduction. Both hits exhibited activity against SARS-CoV replication in a confirmatory $TCID_{50}$ assay. At a survey concentration of 10 μM, SR-36097 potently diminished SARS-CoV infectious yields in Vero E6 (3 log_{10} PFU titer reduction) and primary HAE cells (2 log_{10} PFU titer reduction) in testing performed at VUMC and UNC, respectively **(Fig. 2).** SR-36100 also potently inhibited SARS-CoV infectious yields in Vero E6 cells (2 log_{10} PFU titer reduction). Neither SR-36097 nor SR-36100 showed activity against MERS-CoV in HAE cultures. Cytotoxicity of SR-36097 and SR-36100 in Vero E6 (ATP levels) and HAE (RNA expression levels of apoptotic signature genes) was insignificant at concentrations ≤10 μM. Taken together, these initial results demonstratate that two SR HTS hits function as potent inhibitors of SARS-CoV replication and warrant further testing for SAR, and testing with other CoVs.

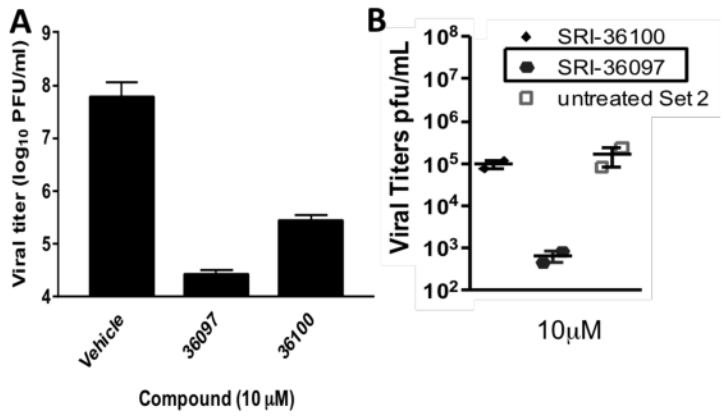


Fig. 2. Effect of SR anti-CoV lead compounds on replication of SARS-CoV in Vero E6 (A) and HAE (B) cells. 36097 demonstrated potent antiviral activity in both culture systems.

New Lead Compound. With the surprising success of the nucleoside analog GS-5734 broadly against WT CoVs that encode the proofreading exonuclease, and with the advancement of the GS-5734 in testing and follow on funding, we thought it important to test additional nucleoside analogs that might show broad and potent activity against WT CoVs. EIDD-1931 is a cytidine analogue (Mutation Research. 1980. 72:43) with broad-spectrum antiviral activity and known activity against the alphaviruses Chikungunya and Venezuelan equine encephalitis viruses, as well earlier studies indicating some activity against CoVs but with no development. We requested and obtained the compound from the Emory Institute for Drug Development for testing in our program. We tested EIDD-1931 against group 2a (MHV), 2b (SARS-CoV), and 2c (MERS-CoV) β-CoVs at VUMC and UNC. EIDD-1931 demonstrated profound viral inhibition, with EC_{50} values ranging from 0.004μM to 0.4μM in transformed cells and primary human airway epithelial cultures without demonstrable cytotoxicity, as well as potency with >3 log reduction in virus titer. We performed preliminary studies with EIDD-1931 against remdesivir (GS-5734) resistant mutants. Surprisingly, the mutations resulted in increased viral sensitivity to the unrelated cytidine analog EIDD-1931, suggesting that GS-5734 resistance does not generalize to other nucleoside analogs and that EIDD-1931 may interfere with CoV replication through a different mechanism involving the viral RdRp. They further support the possibility that combinations of different nucleoside analogs might be possible to for synergistic inhibition, limiting emergence of resistance and broadening spectrum.

B.4. Opportunities for Training. Graduate students are active in the project at Vanderbilt and UNC. Individual development plans (IDPs) are generated on an annual basis for all graduate students. These are used for defining key objectives and goals and reviewed on at least an annual basis. For the AD3C program, IDPs include specific goals relevant to the project. IDPs assist in analysis of progress and future training and career development. Construction of the IDP includes creation, review, and updating of biosketches and CVs; these serve as learning tools for presenting professional training and accomplishments in formats relevant to research funding proposals.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: VANDERBILT UNIVERSITY MEDICAL CENTER

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	(b)(6); (b)(3):7 U.S.C. § 8401				Project Leader	(b)(4); (b)(6)				37,712.00	4,525.00	42,237.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **42,237.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
5	1 Res. Asst., 2 Res. Prof., 1 Lab Mgr., 1 Sr. Res Spec.	(b)(4)			107,284.00	25,751.00	133,035.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**133,035.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**175,272.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: VANDERBILT UNIVERSITY MEDICAL CENTER

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: VANDERBILT UNIVERSITY MEDICAL CENTER

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	62,820.00
2. Publication Costs	4,000.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Repairs and Maintenance	10,000.00
Total Other Direct Costs	**76,820.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**256,092.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	58.0	256,092.00	148,533.00
		Total Indirect Costs	**148,533.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**404,625.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: (b)(6); (b)(3):7 U.S.C. § 8401 udget Justification_Yr 5_11.28.17.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION ([redacted] LAB, PROJECT YEAR 5)

PERSONNEL ($144,995.00)

[redacted] **months).** [redacted] will continue to serve as the Principal Investigator of Project 2. [redacted]

[redacted] **months).** Dr. [redacted] of virology experience. He [redacted] is an authorized Select Agent user and will coordinate all investigators and studies in the BSL3. [redacted] will directly perform portions of studies in Aim 1, including additional testing of candidate CoV antivirals from SR and Gilead. [redacted] will oversee all experiments using engineered SARS-CoV and MERS-CoV.

[redacted] **months).** [redacted] of virology experience and will carry a leading role in the laboratory antivirals program. Prior to joining the lab, [redacted] served as a project manager for the [redacted] will oversee and perform studies at Vanderbilt in Aims 1 and 2. [redacted] will work with [redacted] on BSL2 components of studies of MERS-CoV and SARS-CoV. Additionally, [redacted] will interact with collaborators on data analysis, presentation, and publication.

[redacted] **months).** [redacted] has worked with [redacted] has extensive experience in performing mutagenesis and in engineering recombinant viruses using reverse genetics systems for MHV, SARS-CoV, and MERS-CoV. [redacted] will carry out support experiments at BSL2 for Aims 1 and 2, including preparation of mutagenized reverse-genetic plasmids, viral RNA sequencing, and data analysis. She will coordinate all project purchases.

[redacted] **months).** [redacted] joined the [redacted] lab in Aug. 2017. Prior to her graduate studies, she worked several years in the pharmaceutical industry, engaged in multiple facets of drug development and product processing. She has extensive experience in biochemistry, molecular biology, microbiology, and *in vitro* cell culture systems. [redacted] will conduct experiments supporting Aims 1 and 2 at BSL2. She is completing training as a Select Agent user and will work with [redacted] to perform studies at BSL3 using MERS-CoV and SARS-CoV.

Research Assistant TBD [redacted] months). This individual will perform work supporting Aims 1 and 2, including quantitative testing of CoV-active lead compounds and structural analogues at BSL2; selection, genomic analysis, and phenotypic studies of drug-resistant MHV mutant viruses; assistance of [redacted] in the engineering of recombinant Coves using reverse- genetics techniques; and preparation of cell cultures and reagents for experiments performed at BSL3.

FRINGE BENEFITS ($30,277.00). Fringe benefit calculations are derived from the current Vanderbilt University Medical Center guidelines.

LAB SUPPLIES ($62,820.00)

Cell Culture Supplies, Serum, and Media ($14,000). A large amount of cell-culture work is associated with the project, requiring media, serum, and sterile plastic ware. Consequently, funds are requested for media,

serum, and related cell culture supplies to maintain multiple cell lines and measure a variety of virus replication parameters. Reagents for the extended passage of SARS-CoV and MHV in the presence of nucleoside analogs and lead compounds will be required to test for development of resistance in Aim 1.

BSL3 Supplies, Protective Gear, and Disinfectants ($33,820.00). All testing of SARS-CoV and MERS-CoV, including monitoring development of drug resistance and confirmation of lead compounds, will be performed under strict BSL3 protocols. This will include extensive use of plastic ware, tissue culture reagents, and materials for plaque assays and RNA isolation. BSL3 PPE (personal protective equipment) is required for all work done at BSL3, Regular delivery of CO_2 for incubators is also needed, as are materials for autoclaving and disposal of waste. Materials for shipping of samples between UNC and Vanderbilt are required. Dedicated reagents are required for analysis of SARS-CoV RNA at BSL2 because viral RNA is regulated as a Select Agent.

Enzymes and Reagents ($15,000). Engineering mutations within plasmids carrying fragments of viral genomes will require enzymes and reagents specified by the corresponding protocols. Assembling recombinant MHV, SARS-CoV, and MERS-CoV requires large amounts of expensive restriction enzymes (e.g., BsmBI) and DNA ligase. Molecular biology grade agarose, DNA purification kits, and DNA markers are needed for identifying and isolating assembly intermediates to generate full-length cDNA clones of recombinant viruses. In addition, recovery of viruses from cloned cDNAs requires expensive T7 RNA polymerase-driven *in vitro* transcription kits and electroporation supplies for introduction of infectious full-length viral RNA transcripts into susceptible cells. Substantial amounts of real-time PCR supplies and reagents (e.g., reaction kits and fluorescent probes) are required to support various experimental procedures proposed in Aims 1and 2. Basic chemicals and buffers are needed for general laboratory techniques. Cytotoxicity kits (e.g., CellTiter-Glo) as well as the 96-well plates and other plastic ware will be used to examine toxicity of lead compounds identified in Aim 1.

REPAIRS AND MAINTENANCE ($10,000): This category includes annual decontamination and complete recertification of the BSL3 lab, which includes required maintenance of any BSL3 equipment and service contracts on major equipment.

PUBLICATIONS ($4,000): Sufficient for two 1-2 publications per year.

TRAVEL ($4,000): These funds will allow travel for participating investigators (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 to UAB for the annual CETR meeting**.** Budgeted amount will allow partial support of travel for two investigators to attend one meeting per year for presentation of scientific results. Relevant venues include the American Society for Virology and the International Congress on Antiviral Research, which is an international meeting in 2018.

A. COMPONENT COVER PAGE

Project Title: Project 3.1 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

STREBLOW, DANIEL N

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphavirus species derive evolutionarily from the New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinctive virus-dependent pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication. This demonstrates that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA-dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds directed against these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, “hits” will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50, CC50, and selective indexes.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify: 1) Breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) Cell type-specificity (biologically relevant cells); 3) Targets of antiviral compounds; and 4) Ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds.
Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 3 B.2 Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Project 3 B4 Streblow.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

1. Quinolinones (SR-33394): This compound series inhibits an early step in the viral lifecycle before or during viral RNA synthesis. We will define the mechanism of action for this class of compounds using in vitro assays. In addition, we will develop assays to assess nsp2 helicase and protease activities in an effort to define SR-33394 mode of action and help drive future HTS efforts. We will characterize the SR-34329 resistant phenotype for VEEVTC83 (nsp2-y101c mutation) for resistance against other analogs in this series. We also will assess the impact of quinolinone resistance on viral fitness in vitro and in vivo. SR plans to synthesize a new series of analogs based upon current SAR, which will be tested for in vitro activity against VEEV and other alphaviruses. Further SAR and testing will be dependent upon the results of the new analog series. We are hoping to be able to test the most active quinolinone analog in vivo during the upcoming year. In addition, a manuscript will be submitted describing antiviral activity of this chemical series.

2. Tetralins-BenzoAnnulenes (SR-33366): Additional SAR for this chemical series will be completed to optimize activity with stability and bioavailability. We plan to finish mechanism of action (MOA) studies for this compound series utilizing resistance phenotype information, in vitro assays and protein/compound binding. Since this compound series displays activity against a wide range of virus families, we will further characterize this property in order to determine the range of activity. We will continue in vivo pharmacokinetic (PK) analysis to determine formulation, dosing amount, route and timing for efficacy studies in our mouse models of CHIKV infection and disease. Three manuscripts describing this chemical series are in preparation (two describing chemical synthesis strategy and SAR and one describing biological activity for this chemical series), and these will be finished within Q1-2 of the next year. Lastly, we are developing assays to assess macrodomain function (ADP ribosyl hydrolase activity) and utilizing nsp3 macrodomain crystal structure information to guide future screen development (HTS and in silico).

3. VEEV 2015 HTS: Further SAR for SR-36427 will be put on hold for the next year. Pending mode of action studies, a manuscript describing the antiviral activity for this chemical series (approximately 50 analogs) is under preparation and should be submitted early in the next year. SR-36426 displays high activity and low toxicity profiles and SAR performed during the past year was promising with multiple analogs displaying improved activity and solubility. SR will continue to provide the group with additional analogs for this scaffold for SAR. Since SR-36426 is broadly active against 5 different Alphaviruses, we will determine the breadth of activity against other viruses. MOA studies should be completed for SR-36426. PK analysis will be performed on the most active compounds. If a highly active, soluble SR-36426 analog demonstrates good PK qualities then it will be tested in mice for activity against VEEV and CHIKV.

4. CHIKV 2015 HTS: SR-36767 & SR-33001 are the new leads for CHIKV but both compounds also block a number of other Alphaviruses. We will continue to perform MOA and breadth of action studies for these two series. SR will continue to synthesize new analogs of SR-33001 in order to optimize compound activity and stability. We hope to perform PK analysis and in vivo testing for these two series during the next year.

5. Project 1, 2, 4 Hits: We will continue to test additional compounds that are active against viruses from the other projects in order to identify broadly active compounds.

B.2 Accomplished under the goals

SPECIFIC AIMS

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 1), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating *in vitro* replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further *in vivo* evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

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Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the VEEV and CHIKV. Following these initial screens, “hits” will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC_{90}, CC_{90}, and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.

Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to virus-specific efficacy and molecular mechanism of action.

Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-viral activity (test multiple Alphaviruses); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of *in vivo* efficacy of lead compounds.

Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.

Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

Progress towards our goals is outlined for each Specific Aim: